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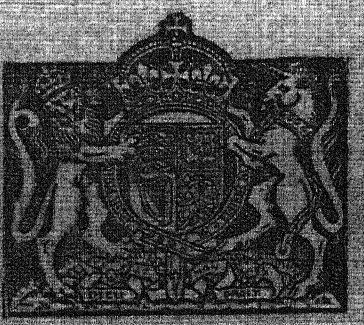

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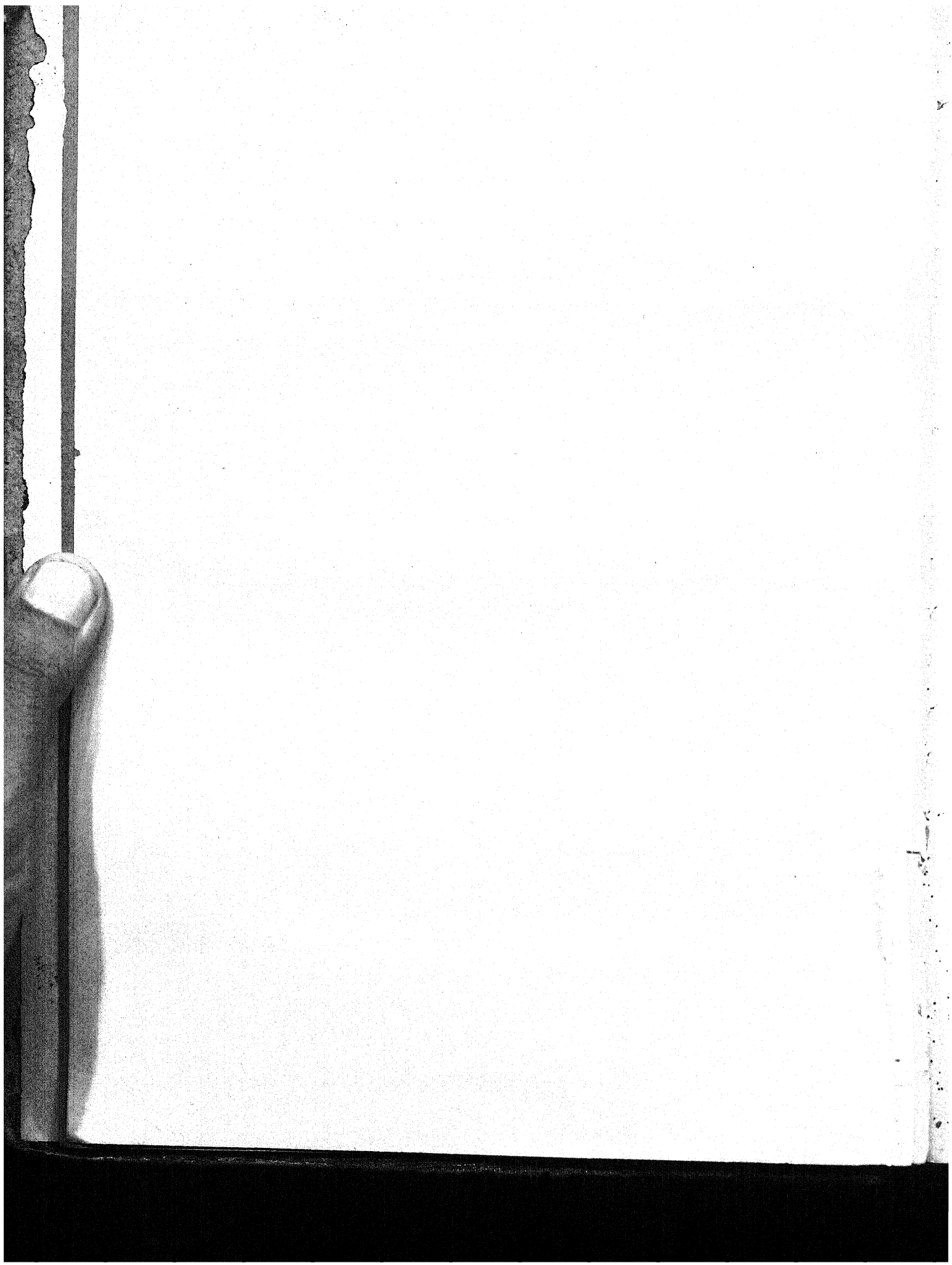

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ORIGINAL ARTICLES

STUDIES IN THE RIND-HARDNESS OF SUGARCANE

I. ANATOMY OF THE STALK AND RIND-HARDNESS

BY

K. L. KHANNA

AND

RAMA RAO PANJE

Sugarcane Research Scheme, Bihar

(Received for publication on 10th May 1938)

(With Plate I and two text-figures)

THE rind-hardness of sugarcane varieties has been a subject of some importance owing to the preference shown by rodents and other gnawing animals to soft-rinded canes, a problem of some concern to growers in many localities. Hard and fibrous canes, while comparatively free from pests and generally good in growth, are not readily accepted by sugar-factories and *gur*-makers, owing to comparative difficulty in milling such canes and their poorer extraction; while soft-rinded canes have to be continually guarded against the depredations of these various kinds of animals. It is intended in these studies to investigate the more important of the factors which influence the hardness of the rind.

These factors are divisible into two categories—the internal and external. The former are mainly anatomical characters which are an expression of the inherent capacity of a variety for the development of a hard rind; the latter are environmental influences which tend to modify, to a greater or less extent, the hardness resulting from the internal characters. These two aspects have been studied separately and this paper deals with the former concerning the anatomical aspect.

I. INTRODUCTION

The anatomical characters of the cane stalk form the primary basis for the development of hardness in the rind. The number of these characters is large and their various combinations appear to produce varying degrees of rind-hardness. The ideal combination of these characters would be such as would result in the development of a hard rind coupled with a soft core. Such a cane would probably be characterised by the exclusive development of those mechanical tissues only which contribute to the hardening of the rind without in any way affecting the core. The present studies were undertaken in order to identify such tissues and the mode of their most effective distribution.

II. Material and methods

The varieties selected for study were Cos 213, 285, 312, 313, 326 and 356. The hardness of the rind in the sampled material of these varieties was determined by an instrument described by Khanna [1935], which measures, by means of a spring arrangement, the force required to puncture the rind with a standard metal point of diameter 0·75 mm. About a dozen canes of each variety were selected at random from the plot and the rind-hardness of these was determined roughly by a few punches. Sub-samples of three canes per variety were selected from among these so that a fair range of hardness of the variety was represented in them ; the actual age of the shoot was not known, but the three selected canes were styled as old, medium and young, according to their hardness, for such they evidently were though not selected as such. From each of these, cross-sections of the ' basal ' joint (the joint just above the one at the ground level) and of the ' dry-leaf ' joint (the one bearing the topmost dry-leaf at the time) were cut after determining the hardness* of each of the selected joints by four punches all round the joint. When the last variety, Co 313, was being tested, unfortunately, the instrument slipped off the cane and the point jammed. As a fresh point would have been sharper and would have given erroneous readings, the old point was ground till it gave the same readings as were taken on the sampled canes before the point got damaged.

The sections were cut with a sledge-microtome out of fresh material, and stained and mounted in Canada balsam. Owing to the presence of hard and soft tissues in the same material, cross-sections less than 60 to 80μ could not be cut without mutilating the sections. In all cases, the sections were taken from the middle portion of the joint. Measurements were made under the microscope at different magnifications. An area of 2·5 sq. mm. of rind 1 mm. thick (radially) in the cross-section was taken for the necessary countings of vascular bundles, etc. The areas occupied by the tissues in the cross-section were measured on camera lucida drawings by means of a planimeter. The degree of lignification was estimated by an ocular micrometer. Considerable difficulty was felt in this determination owing to the thickness of the section, but the measurements were taken at the top focus on the section as accurately as possible, and were used only for their comparative values.

III. Investigations

In a preliminary study [Khanna, 1934] several anatomical characters had been observed and measured where possible, in order to select such among them as were really effective. Among these were :

1. The number of vascular bundles per unit area of the rind in cross-section.

*Thus the readings in the following pages represent the hardness of the particular material under observation and not necessarily the average hardness of the variety as a whole. By this means, errors due to small samples were avoided, though, it must be admitted, the value of the inferences arrived at was thereby slightly diminished for the purpose of application to the variety as a whole.

2. The size of the vascular bundles and the extent of the occurrence of the various sizes.
3. The area of vascular bundles in cross-section.
4. The area of total lignified tissues in the rind in cross-section, and the distribution of these in the outer and inner portions of the rind.
5. Character and degree of lignification of parenchyma in the rind.
6. Degree of lignification of the vascular sheath cells.
7. Rows of sub-epidermal cells and their lignification.
8. Depth of the peripheral chlorenchymatous layer.
9. Tissue tensions, etc.

Only three of these characters (the 1st, the 5th and the 6th in the above list) were found to be of major importance and a detailed study of these was taken up. It was obvious in the course of the study that in any variety, although the various anatomical factors could not operate independently of the related ones, this interdependence or mutual influence was less than could have been expected and that varieties could in most cases be highly partial to some character, to the comparative exclusion of closely related ones.

In the following pages, the three characters mentioned above have been considered one by one, and in the final part their combined effect has been discussed. The relationships are brought out only by arranging the varieties in the descending order of the intensity of the character under question and then finding out how far in a direct relationship the rind-hardness of the varieties so arranged also comes out in the same order and accounting for any possible exceptions. It follows from this that if all the characters, or at least all the major ones, are considered in their combination and the varieties arranged in a certain order of this combined effect, then the hardness of the varieties so arranged would also come out in the same order, and very possibly show a fair arithmetical relationship in ratios. In this manner, the three characters may now be examined individually.

1. Number of vascular bundles per unit area of rind in cross-section

The number of vascular bundles was determined by twelve determinations per variety, six on the basal joint and six on the ' dry-leaf ' joint. The average of these readings, calculated to a percentage of the total for all the varieties in each case, are compared (Table I) with the average rind-hardness of the joints sectioned.

TABLE I

Relation between the number of vascular bundles per unit area of the rind in cross-section, and the rind-hardness

'Dry-leaf' joint			'Basal' joint		
Variety	Average No. of vascular bundles in 1 sq. mm. of rind in cross-section (per cent of total)	Rind-hardness in lbs.	Variety	Average No. of vascular bundles in 1 sq. mm. of rind in cross-section (per cent of total)	Rind-hardness in lbs.
Co 356	21·8	18·3	Co 356	21·0	24·4
Co 285	17·8	13·0	Co 213	17·6	18·3
Co 213	16·4	11·7	Co 313	17·1	17·0
Co 312	15·5	10·4	Co 312	15·7	16·5
Co 313	18·3	13·7	Co 285	15·0	20·2
Co 326	13·6	16·8	Co 326	13·5	25·0

The close relationship between the number of vascular bundles and the rind-hardness in the case of the 'dry-leaf' joint as well as in the basal joint may be made out from the above table. Co 326 appears as a striking exception in both sections of the table, and Co 285 in the case of the basal joint alone. In these varieties, the hardness is greater than is suggested by the number of vascular bundles in them, and this, as will be seen later, is because these varieties lignify the parenchyma of their rind-region to an unusually high degree (Plate I, fig. 1). It will be noticed that Co 326 does so even at an early stage. The relationship is clearer in the case of the 'dry-leaf' joint, because lignification is usually a later development and does not seriously interfere with the influence of the other characters which are operative at that stage.

2. Lignification of the cell-walls in the vascular sheaths

The lignification of cell-walls in the vascular sheaths is one of those characters, which would appear to have a somewhat direct influence on the hardness of the rind. But as a matter of fact, it can be comparatively ineffective if it is not accompanied by its companion-character, *viz.*, the lignification of the walls of parenchymatous cells in-between the outer rows of vascular bundles. The two characters do not necessarily appear together or develop to the same

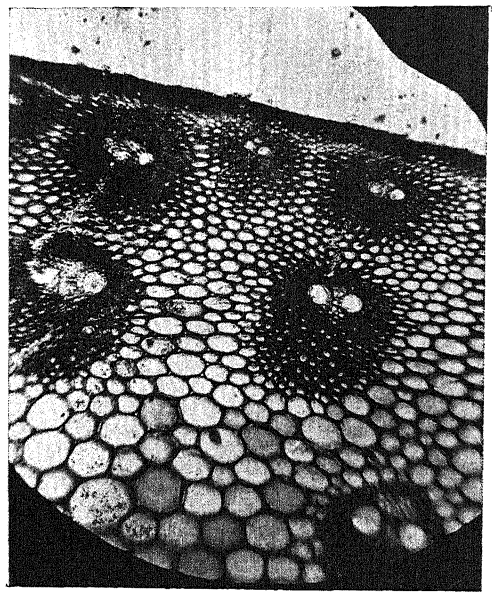


FIG. 1

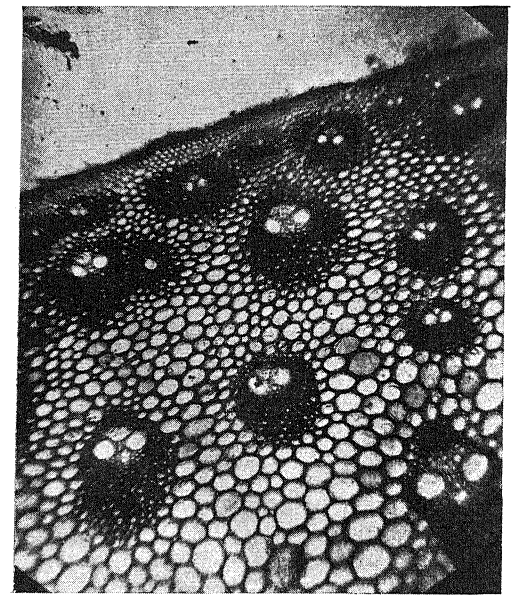


FIG. 2

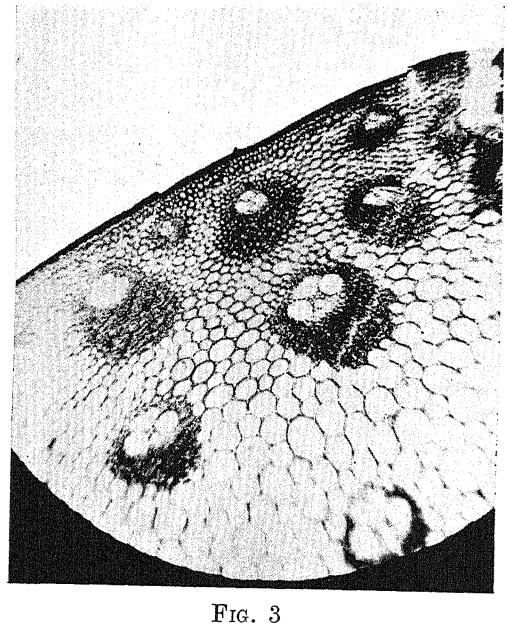


FIG. 3

FIG. 1 : Part of a cross-section through the rind portion of Co 326, showing the strong lignification of the rind tissues in general, and of the inter-vascular parenchyma in particular. The larger size of the vascular bundles, in comparison with those of Co 356 and Co 313 (FIGS. 2 and 3), is also noteworthy. FIG. 2 : Part of cross-section through rind portion of Co 356. The inter-vascular parenchyma shows adequate lignification within the rind portion. The vascular bundles are smaller in size and are more aggregated towards the periphery than they are in Co 326. The rind structure in Co 356 is thus more compact, the hardness of the rind having been attained by an "economic" distribution of lignifying tissues in the rind, rather than by any extensive lignification of parenchyma or vascular bundles as is the case in Co 326. FIG. 3 : Part of cross-section through rind portion of Co 313, showing the exclusive lignification of the vascular sheath. The inter-vascular parenchyma is poorly lignified. (*Cf.* Figs. 1 and 2). (Microphotographs, magnif. 40 dia.)

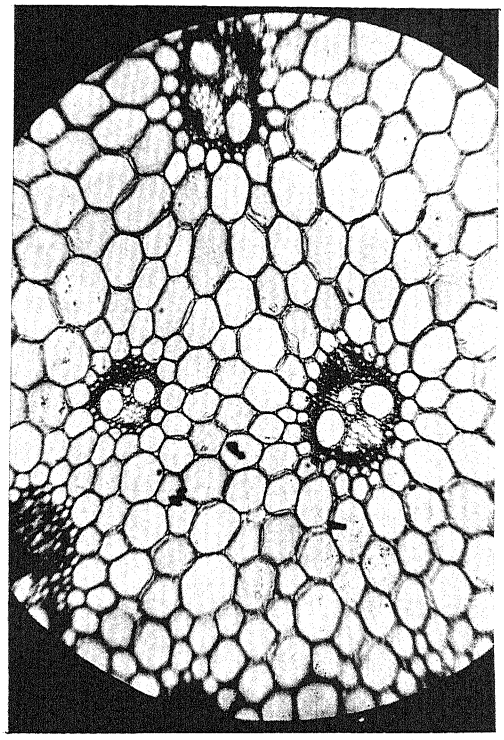


FIG. 4

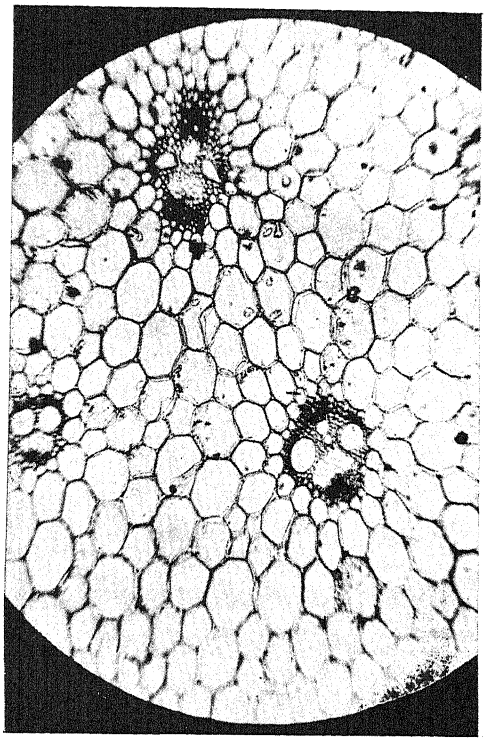
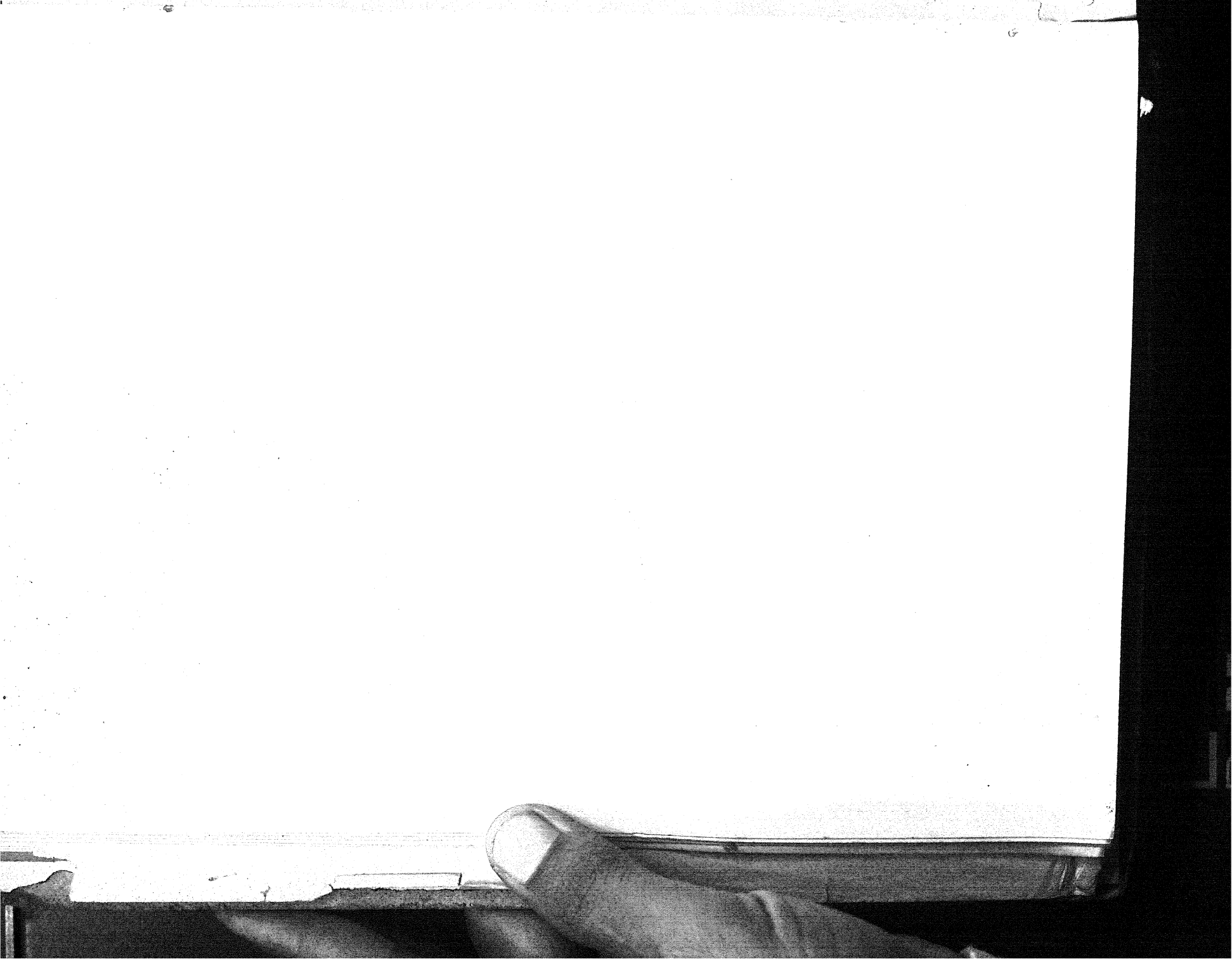


FIG. 5

FIG. 4 : Part of cross-section through stalk of Co 326, showing the maintenance of considerable lignification in the parenchyma in the interior of the stalk beyond the rind portion. This maintenance of lignification in the interior obliterates the clear demarcation of the rind in Co 326. FIG. 5 : Part of cross-section through stalk of Co 356. The lignification of the parenchyma in the interior is comparatively less than that in Co 326. The relegation of lignified tissues to the extreme periphery to form a hard and well-marked rind in Co 356 leaves the parenchyma in the interior almost unlignified, causing thereby comparatively a low fibre content in the variety. (Microphotographs, magnif. 40 dia.)



degree and this fact makes it necessary to consider them separately though they are related to and are the manifestations of the same phenomenon—the lignification of the cell-walls in the rind.

The thickness of the cell-wall (Table II) is calculated to a percentage of the total in all the varieties. Each of the figures is derived from thirty-six readings on the sheath-cells.

TABLE II

Relation between the lignification of sheath-cells of vascular bundles in the rind and the rind-hardness

'Dry-leaf' joint			Basal joint		
Variety	Lignification in sheath-cells in rind (per cent of total)	Rind-hardness in lbs.	Variety	Lignification in sheath-cells in rind (per cent of total)	Rind-hardness in lbs.
Co 326	20·8	16·8	Co 326	23·1	25·0
Co 285	18·3	13·0	Co 285	19·1	20·2
Co 313	18·1	13·7	Co 313	16·1	17·0
Co 356	15·6	18·3	Co 312	14·4	16·5
Co 312	15·1	10·4	Co 213	13·7	18·3
Co 213	12·2	11·7	Co 356	13·5	24·4

This table again shows a clear relationship between the two characters, though it includes two outstanding exceptions, Co 356 and Co 213. These two varieties appear harder in their rind than can be judged from the lignification of their vascular sheaths, and the reason is that in these two varieties, and particularly in Co 356, the number of vascular bundles is a predominating character and the varieties are more or less correspondingly weak in the lignification of the vascular sheath. A close scrutiny of Tables I and II will bear out this fact and will further show that the higher a variety occurs in Table I, the more gravely does it depart from the expected estimate of its rind-hardness in Table II. Thus, Co 356 shows unusually high numbers of vascular bundles (Table I) and the lignification of the vascular sheath being in it weaker than would be normal for it, it appears as strongly exceptional in Table II. Co 213 is less so in this respect.

3. Lignification of the inter-vascular parenchyma

The lignification of the inter-vascular parenchymatous cells is not perhaps as striking a character of the stem anatomy of sugarcane as it is in many kinds

of grasses but it is a character of considerable importance in the development of hardness in the rind. The mechanical principles involved in the development of strength or hardness in a structural frame work of this kind are too well known to need a description.

The percentage figures (Table III) are derived from averages for corresponding varieties, and each is based on thirty-six determinations. The comparative degree of lignification, as in other characters tabled above, is expressed as percentage of the total. As the character is developed to a significant extent a considerable time after the development of the joint, it has been worked out and compared only in the basal joint, where it finds its fullest expression.

TABLE III

Relation between the lignification of the inter-vascular parenchymatous cells and the rind-hardness

Variety	Basal joint	
	Lignification of the inter-vascular parenchyma (per cent of the total)	Rind-hardness in lbs.
Co 326	23·5	25·0
Co 285	18·7	20·2
Co 356	17·9	24·4
Co 312	14·5	16·5
Co 213	13·9	18·3
Co 313	11·5	17·0

The order in which the rind-hardness figures appear in Table III is at first sight irregular, but here again a consideration of the two characters already dealt with will show that the exceptions in the list go to prove the rule, for it is easy to see that two important exceptions, Co 356 and Co 213, owe the high values of their hardness, as had been mentioned before, to the predominance in them of character I. Co 313 departs from the expected rind-hardness value to a slight extent as it is partial to the lignification of the vascular sheath, which is its predominating character (Table II and Plate I, fig. 3) and possibly also to some other subsidiary characters such as sub-epidermal cell-wall thickening, etc. Thus when the 'exceptions' are properly accounted for, the general agreement between the character and the rind-hardness becomes evident in all cases.

IV. Discussion

Before evolving the resultant effect of the three factors, it is necessary to understand their inter-relationship and to satisfy oneself of the independent operation of each. In a biological system involving the closest co-operation of tissues and functions, it would be unreasonable to expect a totally independent development of the different components contributing to a common ultimate end and, to some extent, the components will undoubtedly influence each other. But as it happens, the components are really more independent in their operation than can be expected, and this fact has been clearly brought out in the three characters under which the varieties have been considered. Each of the tables in the previous section shows close general agreement between the intensity of the character and the rind-hardness, but a deeper significance comes to be attached to the ' exceptions ' which have been pointed out in each case ; for the turns which the varieties take in assuming the role of exceptions in the successive tables is in itself an indication and a natural consequence of the differential predominance of the characters operative in them. In other words, the varieties which come out as exceptions when their weaker character* is being considered, will naturally come out in the normal sequence when their predominating character is tabled out, and the fact that the same variety does not always stand out as an exception shows that different characters predominate in different varieties in contributing to the hardness of the rind.

The relationships of the characters may now be considered.

1. Number of vascular bundles.—Of the characters examined, this appears to be the most independent one and though it shows considerable variation within a variety, it is not subject to variations in the course of the ontogenic development ; in this respect, it differs from developmental characters like lignification, etc. Indeed, although the present data is insufficient to venture an assertion on the point, it would not be unreasonable to class the density of aggregation of vascular bundles as a varietal character, if varieties show significant differences.

The character, however, is really a part of a greater complex, namely, the actual quantity of the vascular strand element in the body of the cane or its rind. Variation in this complex depends upon the variation in its three independent components—the number, the size and the degree of lignification of the vascular bundles. Of these, the first and the third are studied here as major characters, the second does not appear to have as great an effect on the rind-hardness as the other components, though its variations bear materially upon the vascular density. In the ' dry-leaf ' joint, its variation among the varieties is poor ; in basal joints, its effect is easily masked by lignifications,

*By a ' weaker ' character is here meant the character which does not contribute mainly or largely to the hardness of the rind in that variety, and by a ' predominating ' character is meant the character which does so.

and when varieties (Cos 312, 313, and 213 in Table IV, basal joint), where lignification does not predominate are considered the variation ascribable to the size of the bundle is found to be very poor, so that the character as a whole seems to be far less effective than the other two components.

TABLE IV

Relation between the size of the vascular bundle and the rind-hardness

'Dry-leaf' joint				Basal joint			
Variety	Average area of vascular bundles in 1 sq. mm. of rind in cross-section in sq. mm.	Average area of each vascular bundle in sq. mm.	Rind-hardness ascribable to one vascular bundle per sq. mm. of rind in cross-section in lbs.	Variety	Average area of vascular bundles in 1 sq. mm. of rind in cross-section in sq. mm.	Average area of each vascular bundle in sq. mm.	Rind-hardness ascribable to one vascular bundle per sq. mm. of rind in cross-section in lbs.
Co 326	0·306	0·059	3·231	Co 326*	0·382	0·090	5·859
Co 313	0·317	0·052	2·233	Co 312	0·421	0·080	3·133
Co 312	0·308	0·051	1·714	Co 285*	0·432	0·081	3·789
Co 285	0·346	0·048	1·806	Co 213	0·382	3·064	3·050
Co 213	0·304	0·047	1·809	Co 313	0·355	0·063	3·000
Co 356	0·369	0·042	2·095	Co 356*	0·372	0·055	3·588

*Varieties with highly lignified tissues in the rind.

2. Lignification of the vascular sheath and of the inter-vascular parenchyma.— These two characters are related to one another in the sense that they are the manifestations of the same developmental phase. The relationship is also noticeable in the varieties, for, as a rule, the lignification in these proceeds proportionately, though not simultaneously, and varieties showing a high development of the one also show a corresponding development of the other. This, however, is true only as a general rule, for these very characters afford a striking illustration of the partiality shown by varieties (Table V) to any one of the two related characters or to any part of a general character.

TABLE V

Relation between the lignification in the vascular sheath and in the inter-vascular parenchyma

Variety	Lignification in vascular seath (per cent of total)	Lignification of inter-vascular parenchyma (per cent of total)
Co 326	23·1	23·5
Co 285	19·1	18·9
Co 313	16·1	11·5
Co 312	14·4	14·5
Co 213	13·7	13·9
Co 356	13·5	17·9

It will be noticed that while there is in general a relationship between the two characters, two varieties, Co 313 and Co 356 (compare figs. 3 and 2 in Plate I), show a strongly partial development, the former for the lignification of the vascular sheath and the latter for that of the inter-vascular parenchyma. This occasional partiality of certain varieties which can show a perceptible predominance to a part of a general character, makes it necessary to consider these two characters independently.

3. Number of vascular bundles and the lignifications of the inter-vascular parenchyma.—These two by nature of their case, as well as by experimental evidence, have very little inter-relationship. The increase in the number of vascular bundles is generally associated with a reduction in the size of the bundle, so that the possibility of the parenchyma being unduly influenced is obviated.

4. Combined effect.—The characters have been examined in the light of the contribution of each individual character, but in combining them for their resultant effect, one is at a loss to ascribe any proportion for their respective representation. The inequalities due to the characters being expressed in different units are eliminated by reducing the contributory values in each variety to a percentage of the total for all the varieties, thus establishing them on a basis of numerical equality in range. But any assumption on the specific contributory value of each character as a whole can only be based on a theoretical reasoning as no experimental evidence can be obtained on it, and any judgment on the point will naturally be arbitrary.

The first character, the number of vascular bundles per unit area in a cross-section is an independent character; it is in a sense an expression of the quality of the rind as a whole, and not, as in other cases, a quality of particular parts of the rind. In forming a combination with this character, therefore, the other two characters cannot be taken on an equal basis of representation, as they are applicable to only certain parts of the rind-structure. There are two ways in which the other characters may be represented. In the first, one may consider the lignification of the inter-vascular parenchyma as a universal development in all the tissues of the rind, and that of the vascular sheath as an additional and earlier development which is restricted to particular areas. Under this assumption, the average area of the vascular tissues being about two fifths of the total area of the rind in cross-section (Table VI), one may represent the character, *viz.*, the lignification of the vascular sheath at 40 per cent of its value, and the other two characters at their full value.

TABLE VI

Average area occupied by vascular bundles in a cross-section of the rind of the basal joints

Variety	Average percentage area of vascular bundles in a cross-section of the rind
Co 285	43·2
Co 312	42·1
Co 326	38·3
Co 213	38·2
Co 356	37·2
Co 313	35·5
Average percentage area in all varieties	39·1

In the alternative, one may consider these lignification-characters as affecting different portions of the rind, and represent them in the proportion of the areas in which they are effective, 40 per cent in the case of one and 60 per cent in the case of the other. The combined effect has accordingly been calculated on these two bases, and the percentage values (Table VII) obtained from this are compared with the rind-hardness.

TABLE VII

Relation between the combined effect of the anatomical characters and the hardness of the rind

Variety	Combination of characters*		Rind-hardness	
	$A+\frac{2B}{5}+C$	$A+\frac{2B+3C}{5}$	Actual in lbs.	Percentage of total
Co 326	19·3	18·4	25·0	20·6
Co 356	18·5	18·6	24·4	20·1
Co 285	17·2	16·9	20·2	16·6
Co 213	15·4	15·7	18·3	15·1
Co 313.	14·6	15·2	17·0	14·0
Co 312	15·0	15·1	16·5	13·6

*A : The number of vascular bundles per unit area of the rind in cross-section.

B : The degree of lignification of the cell-walls of the vascular sheath in the rind.

C : The degree of lignification of the cell-walls of the inter-vascular parenchyma in the rind.

All the figures in the 2nd, 3rd and 5th columns are expressed as percentages of the total of all the varieties. The rind has throughout been taken at a thickness of 1 mm.

The agreement of either of the calculated combinations with the rind-hardness is very close in the above table and also in the graphical representations (Figs. 1 and 2), indicating that the three major characters studied here account for by far the greatest part of the hardness of the rind in the six varieties taken up. It cannot of course be claimed that these are solely responsible for the hardness, for there are many subsidiary characters apart from the environmental influences which, though not actually concerned in this function, may yet have some influence on the hardening of the rind, or the softening of it. In addition to these subsidiary characters, there may be other internal influences, such as the moisture-content of the cane, the extent of pith and cavity development in extreme cases, and possibly also the morphological characters such as the girth and the shape of the joint. But comparatively, their effect seems to be much less than that of the major anatomical characters.

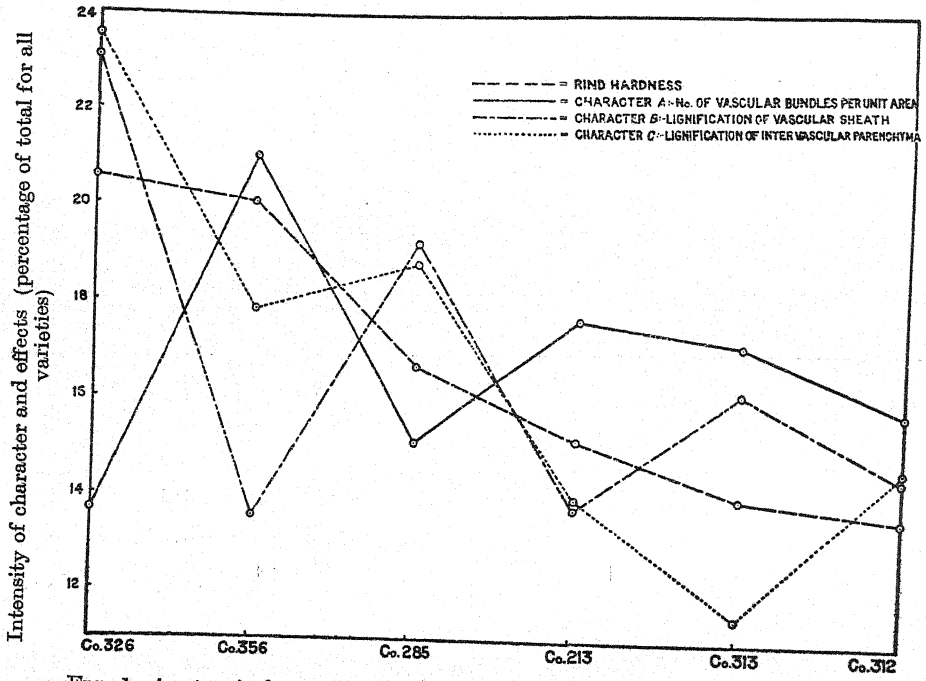


FIG. 1. Anatomical characters and rind-hardness of sugarcane varieties (individual effect)

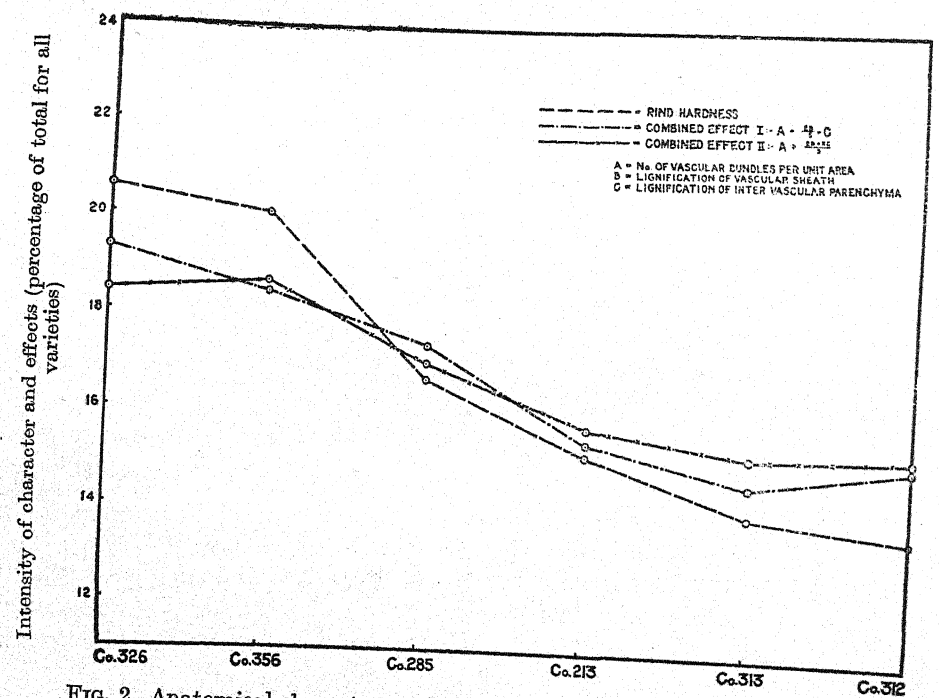


FIG. 2. Anatomical characters and rind-hardness of sugarcane varieties (combined effect)

5. Rind-characteristics in the varieties.—It has been seen that varieties differ from each other in the differential predominance of characters in them and this fact has given some insight into the characteristics of the rind of the varieties. The study having been on small samples, it would not be possible to give a generalized account of these characteristics, but the outstanding features may be examined.

A variety which does not show any outstandingly predominant character is Co 313. It does not even show a clearly marked rind, the vascular bundles being distributed somewhat uniformly. Lignification is poor in the parenchyma, but the vascular sheaths are well lignified (Plate I, fig. 3). The variety shows some resemblance to the softer types of cane.

Co 312 resembles Co 313 in the absence of a sharply-marked rind. The lignification is fair and maintained well into the interior.

Co 213 has a sharply marked rind, the size of the bundles is small, which seems to be a desirable character but the distribution is not as close as in Co 356.

Co 285 is a lignifying cane, both the vascular bundles and the inter-vascular parenchyma lignifying to a high degree. This is reflected in the high value it shows in fibre determinations.

Co 326 and Co 356 are perhaps the most interesting varieties of the series, presenting as they do diametrically opposed tendencies. Co 326 has few and large bundles, but its inter-vascular parenchyma is strongly lignified, the lignification extending so far into the interior as to obliterate the demarcation of the rind and to cause a high fibre-content (Plate I, fig. 4). Co 356, on the other hand, is characterised by an unusually large number of small bundles, well aggregated towards the periphery and cemented together by a sufficiently high lignification of the parenchyma, thereby presenting a comparatively thin but well-marked rind (Plate I, figs. 2 & 5). It has developed the rind-features on what may be called economic lines, and this is well brought out by the fact that though it has a hard rind, its fibre value is comparatively low. Altogether, Co 356 is nearest to an ideal type of cane, as far as the characters in question are concerned, but the fact that it is an intergeneric hybrid between sorghum and sugarcane is not without a significance in this connection.

V. Summary

In an anatomical study on the hardness of the rind in sugarcane varieties, three factors—the number of vascular bundles per unit volume of the rind, the lignification of the cell-walls of the vascular sheaths and the lignification of the inter-vascular parenchyma—were found to contribute in the greatest measure to the hardness of the rind. The degree of development of these characters was found to differ among the varieties, one or the other of the characters

predominating in each of the varieties in contributing to the hardness. Their combined effect, calculated on reasonable proportions of representation for each of the characters, was found to agree closely with the resultant hardness, as determined by the rind-hardness machine.

A few outstanding anatomical characteristics of the rind in different varieties are given, and the features of a desirable type of rind anatomy are demonstrated in one of the varieties.

VI. Acknowledgment

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A STATISTICAL STUDY OF THE LOSS CAUSED BY BORERS AND TERMITES TO MATURE SUGARCANE AT PUSA IN 1935 AND 1936

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(With one text-figure)

Introductory

For the last ten years or so observations on the incidence of borers, termites and other pests of sugarcane have been taken at Pusa and some other entomological stations in India. As most of the workers have studied only the extent of the incidence of the pests, the impression prevails that this study gives a full idea of the amount of damage caused by them to the crop. A little consideration will make it clear that if a crop shows an incidence of stem borer of say 12 per cent, it does not necessarily mean that the crop would suffer an actual loss of 12 per cent in weight or sucrose content. Moreover, if a crop is infested by the stem-borer it may not suffer the same amount of loss as the one infested to the same extent by the top-borer, inasmuch as the two pests are quite distinct from each other in their biology and method of attack. Obviously the real criterion of the loss is the decrease in weight of the infested canes and the deterioration in the sucrose content of their juice. The published records * show that while some observations on the weight and sucrose content of canes infested by termites and borers (stem-,top- and root-borer considered together) have been taken on small number of canes at several places in India, hardly any critical study, based on statistical analyses, of the actual loss caused by various pests in the weight and quality of juice of the infested cane has been made in this country. Recently Haldane [1937] has examined a large number of canes from this point of view but his work also is not based on statistical analyses and furthermore he did not differentiate the various borers from one another. We therefore do not yet know the extent of loss caused by different borers individually. The importance of such a study to entomologists as well as to students of agriculture need hardly

* Annual Reports of the Imperial Entomologist for 1931—36, of the Director of Agriculture, Punjab for 1932—36, of the Director of Agriculture, U. P., for 1935-36.

be emphasized. At Pusa in March 1935, a large amount of data, which could be examined statistically, were collected on the variety Co 331, in reference to the loss in weight caused by various pests individually and in combination with one another. The juice of a number of infested canes was analysed to determine the extent of deterioration caused by different pests. In March 1936, five varieties of cane, *viz.*, Cos 331, 313, 299, 231, 213, which had been grown in randomized blocks, were similarly examined. The results of studies made at the two harvests are summarized in the following pages.

The following are the most common species of pests met with :—

Top-borer—*Scirpophaga nivella* Fab. A few specimens of *Scirpophaga monostigma* Zell. were sometimes obtained.

Stem-borer—*Diatraea* (=*Argyria*) *sticticraspis* Hmp., about 85 per cent ; *Diatraea venosata* Wlk., about 12 per cent ; and *Chilo zonellus* Swinh. and others about 3 per cent.

Root-borer—*Emmalocera depressella* Swinh.

Termites—*Odontotermes assumthi* Holmgr.

Material and methods

The crop of Co 331 studied in March 1935 (harvest time) was about one acre in size and was growing in the Gonhri field of the Pusa Estate. The soil of the field was heavy and clayey and the crop was good on the whole. A set of eight plots, each measuring 121 ft. × 6 ft. and having two rows of canes, was selected at random. All canes in the selected plots were uprooted, the pests infesting them, if any, were examined and the canes were sorted out on the basis of infection by different pests. The number of canes in each set and their weight were noted down. The detailed data are given in Appendix I. Again, in the Spring of 1936 similar observations were taken on five varieties, which had been specially planted for the purpose. The soil in this field was clayey and fairly heavy. The field (2·5 acres) was divided into eight blocks and each block was sub-divided into five plots, each measuring 80 ft. × 24 ft., with a six feet passage all round. Each plot contained one particular variety and the planting was done in such a way that each block contained all the five varieties, labelled A, B, C, D, E (Fig. 1). Planting was done in all plots lengthwise (east to west) in rows, three feet apart from one another. Thus there were eight rows of each variety in a plot and the various varieties were distributed at random in each block. At the time of harvest two rows, excluding the border rows, were selected at random from each plot and all the canes therein were uprooted and sorted out in accordance with the pests infesting them and their weight noted. As far as possible the canes were cut and topped at a uniform level. The detailed data are presented in Appendix II, A-E. The percentage number of canes attacked by different borers individually or in combinations, the percentage loss in weights of canes infested by various borers as compared with that of canes free from pests and the total loss sustained in the whole plot due to the attack of pests individually and considered together were worked out for each plot, and on the basis

of these figures the mean and the standard errors were calculated for different varieties separately. It may be noted that the total loss in the weight of cane in each plot due to pests was calculated on the assumption that all the canes in a plot would have the same weight as those which were free from the pest attack. The data for 1936 were analysed according to Fisher's method of analysis of variance. In some replications certain combinations of pests were not met with. The loss of weight in such cases being indeterminate, the standard errors were calculated without eliminating the block effects. The method adopted was the same as the analysis of variance which is used when dealing with samples of different sizes for examining the significance between different means.

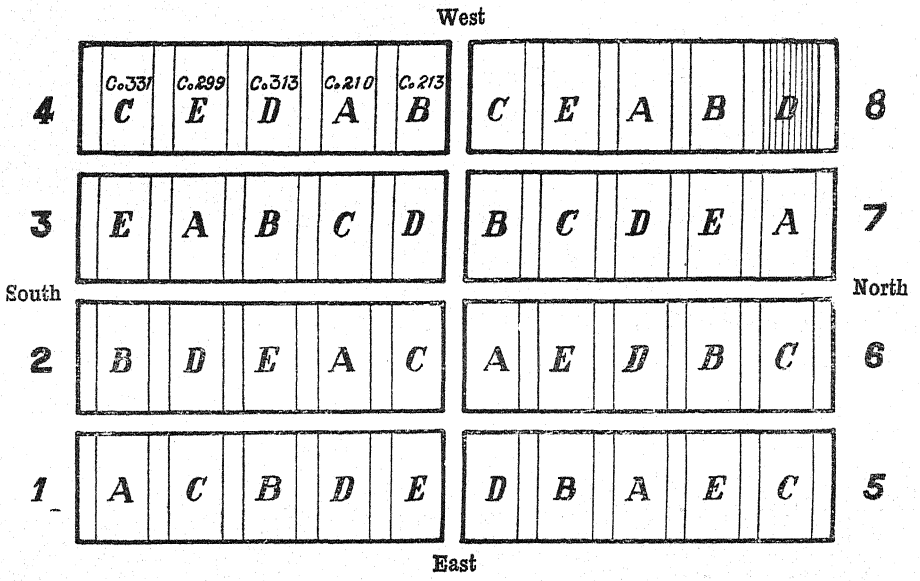

FIG. 1. Lay-out of the experimental blocks in 1935-36

RESULTS AND DISCUSSION

A. Extent of incidence of various pests

The percentages (means of eight replications) of attacked canes of different varieties per unit area by different pests individually and in combination with one another in 1935 and 1936 are given in Table I (column 2) and Table II respectively. In 1935, $22{\cdot}47 \pm 0{\cdot}89$ per cent of canes of Co 331 were entirely free from pests. Of the various borers, top-borer was found infesting the largest number of canes ($20{\cdot}07 \pm 1{\cdot}14$), root-borer came next ($12{\cdot}39 \pm 0{\cdot}48$), and stem-borer was found in $7{\cdot}90 \pm 0{\cdot}50$ per cent canes only. The attacks of all the three borers were significant. Of the various combinations of borers, top- and root-borers were found in the largest number of canes ($28{\cdot}70 \pm 0{\cdot}89$ per cent). The number of canes attacked by termites alone or in combination with different borers was very small, therefore no reliable conclusions about the extent of incidence of this pest could be drawn.

TABLE I

Percentage of infested canes of Co 331 and percentage loss in their weight due to various pests at harvest in 1935

Pests	Percentage infestation	Percentage loss in weight	Remarks
1	2	3	4
Free from pests . .	22·47±0·89	..	
Root-borer . .	12·39±0·48	—23·63±2·54	Attack significant. Increase in weight significant.
Stem-borer . .	7·90±0·50	+31·96±3·05	Attack significant. Loss in weight significant.
Top-borer . . .	20·07±1·14	5·38±3·20	Attack significant. Loss not significant.
Root- and stem-borers .	1·47±0·26	—12·90±6·66	Attack significant. Change in weight not significant.
Root- and top-borers .	28·70±0·89	13·46±2·87	Attack significant. Loss in weight significant.
Stem- and top-borers .	2·29±0·37	24·91±4·03	Attack significant. Loss in weight significant.
Root-, stem- and top-borers	3·63±0·42	17·20±2·48	Attack significant. Loss in weight significant.
Termites . . .	0·27±0·08	—1·02±13·62	Number of canes attacked by termites alone or by termites in combination with other pests too small to lead to any reliable conclusions.
Termites and root-borer	0·15±0·06	—13·48±4·43	
Termites and stem-borer	..	..	
Termites and top-borer	0·38±0·08	36·51±4·05	
Termites, root- and stem-borers	0·01±0·01	—4·44±..	
Termites, root- and top-borers	0·20±0·13	27·67±3·13	
Termites, stem- and top-borers	0·07±0·05	47·31±5·63	
Termites, root-, stem- and top-borers	..		

TABLE II

Percentage of canes attacked by different pests in 1936

Pests \ Variety	Co 331	Co 299	Co 313	Co 213	Co 210	S. E. of mean	S. E. of mean difference	Conclusions as based on analysis of variance
Free from insect pests	40·53	40·65	18·57	31·42	22·37	2·04	2·89	Varietal differences significant.
Root-borer only	16·92	13·66	13·76	9·89	11·12	1·10	1·55	Amount of attack significant, varietal differences also significant.
Stem-borer only	9·40	5·78	13·21	13·96	8·43	1·22	1·73	Amount of attack significant, varietal differences also significant.
Top-borer only	13·92	23·07	13·16	15·43	23·81	1·34	1·90	Amount of attack significant, varietal differences also significant.
Root- and stem-borers	4·74	1·94	11·18	5·43	3·50	0·52	0·73	Amount of attack significant, varietal differences also significant.
Root- and top-borers	7·78	8·89	10·86	6·25	13·73	1·42	2·02	Amount of attack significant, varietal differences also significant.
Stem- and top-borers	2·79	3·69	7·06	7·70	6·10	0·85	1·20	Amount of attack significant, varietal differences also significant.
Root-, stem- and top-borers	1·29	1·58	8·65	4·38	3·64	1·03	1·45	Varietal differences significant; attack on Co 331 and Co 299 not significant.
Termites only	0·94	0·26	0·46	0·59	1·19	0·32	0·45	Varietal differences not significant, attack on Co 299, Co 313 and Co 213 not significant.

TABLE II—*contd.*

Pests \ Variety	Co 331	Co 299	Co 313	Co 213	Co 210	S. E. of mean	S. E. of mean difference	Conclusions as based on analysis of variance
Termite and root-borer .	1·10	0·11	1·03	1·65	1·37	0·24	0·33	Varietal differences significant, attack on Co 299 not significant.
Termite and stem-borer .	0·31	0·04	0·56	0·52	0·59	0·19	0·26	Varietal differences not significant, attack on Co 331 and Co 299 not significant.
Termite and top-borer .	0·10	0·08	0·23	0·32	1·01	0·15	0·21	Attack significant in the case of Co 210 and Co 213 only.
Termite, stem- and top-borers	0·10	0·04	0·23	0·24	0·31	0·12	0·17	Attack significant in Co 210 only.
Termite, root- and top-borers	0·10	0·08	0·23	1·05	1·51	0·27	0·39	Attack significant in Co 213 and Co 210 only.
Termite, root- and stem-borers	..	0·10	0·46	0·72	0·35	0·14	0·19	Varietal differences significant; attack significant in Co 313, Co 213 and Co 210.
Termite, root-, stem- and top-borers	..	0·04	0·38	0·46	0·95	0·17	0·24	Varietal differences significant; attack significant in Co 313, Co 213 and Co 210.

In 1936 (Table II), Co 299 and Co 331 had the highest percentage (about 40) of canes entirely free from pests, Co 313 and Co 210 had the lowest (18·57 and 22·37 per cent, and Co 213 had 31·42 per cent). The varietal differences in this respect were significant. The percentage infestation of top-borer was highest in almost all varieties (13—24 per cent), root-borer came next (10—17 per cent), and stem-borer infested the least number (6—14 per cent) of canes, except in the case of Co 213, in which it attacked more than the root-borer. In Co 313 the three borers were almost equally common. The attacks of the three borers, individually or in combination with one another, were significant in all the varieties except of the combination top + stem + root borers in the case of Co 299 and Co 331. The varietal differences in the attacks of all borers, individually or in combination, were significant.

Regarding the attack of termites alone or in combination with various borers, it was significant generally in the case of Co 210 only. In most cases the varietal differences were insignificant. In fact termites alone or in combination were present in very few cases and therefore no reliable conclusions could be drawn about the extent of their incidence, as was the case in 1935.

B. Comparative loss in weight of cane due to the attack of various pests

The percentages (means of eight replications) of loss in weight of canes of various varieties due to different pests in 1935 and 1936 are stated in Tables I (column 3) and III respectively.

In 1935 when only Co 331 was examined, the loss caused by stem-borer was the heaviest ($31·96 \pm 3·05$ per cent), *i.e.*, if a known number of healthy canes weighed 100 lbs., those infested by this borer weighed about 68 lbs. only. The loss due to top-borer was not significant. Regarding root-borer, it is noteworthy that the weight of canes infested by this borer was higher by $23·63 \pm 1·54$ per cent as compared with that of canes entirely free from any pest. As will be described hereafter, there was also an increase in the sucrose content of the canes attacked by this borer. The losses due to the attack of the three borers in various combinations with one another were significant except in the case of root + stem borer combination.

In the case of termites, alone or in combination with various borers, the loss was insignificant throughout.

In 1936 (Table III), the loss in weight due to top-borer separately or in combination with other borers was the heaviest and significant in all varieties except Co 299. In the varieties in which the loss was significant, it varied between about 16 and 27 per cent. The varietal differences were significant. The loss due to the attack of stem-borer was not significant in any variety, not even in Co 331, in which as stated above this borer caused the heaviest loss in 1935. The loss due to root-borer was significant in the case of Co 210 and Co 313 only. This year, unlike that in 1935, neither the canes of Co 331 nor of any other variety infested by this borer weighed heavier than the healthy canes. The losses on account of the three borers in combination with one another were significant in the case of all varieties except Co 299, but the varietal differences on the whole were not significant.

TABLE III

Percentage of loss in the weight of canes due to different pests in 1936

Variety / Pests	Co 210	Co 213	Co 299	Co 313	Co 331	Conclusions
Root-borer . .	10·18±4·34	5·46±4·34	8·67±4·34	12·86±4·34	3·01±4·34	Differences between varieties not significant, loss in Co 210 and Co 313 significant.
Stem- borer . .	6·14±4·96	—7·54±4·96	—1·81±4·96	5·80±4·96	6·67±4·96	Loss not significant.
Top-borer . .	18·39±3·09	16·65±3·09	3·26±3·09	16·38±3·09	21·76±3·09	Differences between varieties are significant, loss in Co 210, 213, 313 and Co 331 is significant.
Root- and stem-borers	10·15±1·89	4·51±1·89	0·84±1·89	9·52±1·89	10·63±1·89	Differences between varieties not significant, loss in all varieties except Co 299 significant.

Root- and top-borers	20·65±2·78	22·11±2·78	17·96±2·78	22·32±2·78	26·73±2·78	Differences between varieties not significant but loss significant in all the varieties.
Stem- and top-borers	16·61±4·98	18·74±4·98	3·42±4·98	14·63±4·98	27·02±4·98	Differences between varieties significant at 5 per cent level, loss significant in all varieties except Co 299.
Root-, stem- and top-borers	21·69±6·29	26·12±6·29	5·70±6·29	21·90±6·29	19·62±6·29	Differences between varieties not significant, loss is significant in all cases except Co 299.
Termites . .	10·10±11·26	4·27±11·26	30·45±12·59	5·06±12·59	6·48±10·28	Loss significant in the case of Co 299 only.
Termite and root-borer	16·88±6·30	10·28±6·30	32·97±10·28	9·89±6·73	34·54±6·30	Differences between varieties significant at 5 per cent level, loss in Co 210, Co 299 and Co 331 significant.
Termite and stem-borer	9·55±8·03	28·24±8·03	28·69±16·07	6·84±7·18	13·18±8·03	Differences between varieties not significant, loss in Co 213 significant.

TABLE III—*contd.*

Variety / Pests	Co 210	Co 213	Co 299	Co 313	Co 331	Conclusions
Termite and top-borer	17·66±8·79	14·99±11·63	14·39±16·45	—9·04±10·40	3·16±16·45	Differences between varieties and loss are not significant.
Termite, stem- and top-borers	13·61±14·31	—3·95±20·24	—6·96±28·62	7·17±16·53	7·01±28·62	Differences between varieties and loss are not significant.
Termite, root- and top-borers	27·51±5·45	19·03±5·97	—6·96±13·35	3·21±7·71	—3·33±13·35	Differences between varieties not significant, loss is significant in the case of Co 210 and Co 213.
Termite, root- and stem-borers	4·13±14·17	—4·78±11·57	—7·99±20·04	—2·00±14·17	..	Not significant.
Termite, root-, stem- and top-borers	24·69±8·71	18·67±8·71	60·91±23·05	0·61±11·52	..	Loss in Co 299, Co 210 and Co 213 is significant but differences are not significant.

From the foregoing it will be evident that Co 299 suffered comparatively the least amount of loss in weight due to the attack of various borers separately or in combination with one another.

Regarding termites, when occurring alone they caused significant loss (30·45±12·59 per cent) in the case of Co 299 only. In combination with root-borer they caused significant loss in Co 210, Co 299 and Co 331. When they occurred alone with stem-borer, Co 213 suffered significant loss (28·24± 8·03). Co 210 and Co 213 suffered significant loss when attacked by termites in combination with root and top-borers and with root+top+stem borers. Co 299 also suffered significant loss from the latter combination. But as already stated, the number of canes infested by termites alone or in combination with various borers was very small, therefore no reliable conclusion could be drawn about the loss in weight due to the presence of termites.

In the above paragraphs the comparative percentage losses in weight caused by various pests when occurring alone and in combination with one another have been described. But both from economic and entomological view-points it is also important to ascertain the amount of actual loss caused by various pests individually with known degrees of incidence. For this purpose it is appropriate that only those losses should be compared for which the various borers were responsible when occurring singly, as it is very difficult to assess the amount of loss caused by different borers constituting a combination. The percentages of actual loss in weight caused by different borers, when occurring alone, in various varieties during 1935 and 1936 are given in Table IV. In 1935, when only Co 331 was examined, stem-borer with an infestation of about 8 per cent caused a loss of 2·56 per cent, whereas top-borer with an infestation of over 20 per cent did not cause any significant loss. The weight of canes infested by root-borer was higher by 2·87 per cent. This clearly indicates that the extent of incidence does not give any idea of the loss caused by the pests.

In 1936 when top-borer again infested the largest number of canes of Co 331 as well as of all other varieties under investigation, the loss actually caused by this pest, unlike that in 1935, was heaviest in all varieties. Co 210 suffered the most, having 4·45±0·61 per cent loss, Co 331 came next (3·04± 0·61 per cent), Co 213 showed a loss of 2·49±0·61 per cent, Co 313 had 2·12± 0·61 per cent, whereas Co 299 did not suffer any significant loss.

The stem-borer which infested the least number of canes of all varieties did not cause significant loss to any variety. The behaviour of root-borer was also slightly different from the previous year. Whereas in 1935 the canes of Co 331 infested by this borer weighed more than the healthy canes, in 1936, the canes of this as well as other varieties weighed less but the loss was significant in Co 313 only, being 1·76±0·68 per cent.

TABLE IV

Percentage of actual loss caused by different borers when occurring alone, in the years 1935 and 1936

Year and variety	Top-borer	Stem-borer	Root-borer	Remarks
1935, Co 331	1·02±0·61	2·56±0·33	—2·87±0·24	Loss by stem and gain by root significant.
1936, Co 210	4·45±0·61	0·43±0·47	1·10±0·68	Loss by top alone significant.
1936, Co 213	2·49±0·61	—0·84±0·47	0·59±0·68	Loss by top alone significant.
1936, Co 299	0·67±0·61	0·05±0·47	1·33±0·68	No loss.
1936, Co 313	2·12±0·61	0·87±0·47	1·76±0·68	Loss by top and root significant.
1936, Co 331	3·04±0·61	0·82±0·47	0·19±0·68	Loss by top alone significant.

C. Total loss in weight due to all pests

The total percentage loss in weight of various varieties due to all pests combined was calculated for each plot. The data are summarised in the following statement :—

Year	Varieties					Remarks
	Co 210	Co 213	Co 299	Co 313	Co 331	
1935	..	..	..	..	5·77±1·71	Loss significant
1936	12·81±2·22	9·10±2·22	3·92±2·22	11·62±2·22	7·90±2·22	Loss significant in all varieties except Co 299

From the above it will be observed that whereas in 1935 Co 331 suffered 5·77±1·71 per cent loss, it suffered loss of 7·90±2·22 per cent in 1936. In both years the loss was significant. In the latter year, except Co 299, all other varieties examined suffered significant loss. In the order of loss sustained they could be arranged thus : Co 210> Co 313> Co 213> Co 331> Co 299.

D. Influence of various pests on the cane juice

The chemical analyses of nine samples taken at random of the juice of Co 331 in 1935 and of forty samples of Co 331, Co 313, Co 299, Co 213 and Co 210 in 1936 are given in Tables V and VI respectively.

In 1935 the deterioration in the juice of Co 331 due to the attack of borers was similar to the loss in its weight described above. The stem-borer proved most harmful, as the juice of the canes infested by this borer had 13·07 per cent sucrose content against 16·2 per cent of healthy canes. Those infested by top-borer had 15·50 per cent, whereas those attacked by the root-borer had 17·37 per cent, a little over one per cent more than even the healthy canes. In the case of root-borer the purity of juice was also better, being 89·04 per cent as against 86·77 of the healthy canes. The canes infested by the top and stem-borers in combination had the least sucrose content (10·60 per cent).

TABLE V

Chemical analyses of juice of Co 331 in 1935

No. of sample	Pests	Brix corrected	Percentage sucrose	Percentage glucose	Percentage purity
1	Free from pests . . .	18·67	16·20	0·38	86·77
2	Root-borer . . .	19·51	17·37	0·27	89·04
3	Stem-borer . . .	16·57	13·07	0·61	78·89
4	Top-borer	17·80	15·50	0·42	86·69
5	Top- and root-borers . .	16·67	14·02	0·60	84·24
6	Top-, stem- and root-borers .	15·94	13·06	0·67	81·91
7	Termite and top-borer .	16·77	13·35	1·00	79·78
8	Top- and stem-borers . .	14·00	10·60	0·83	75·70
9	Stem- and root-borers . .	17·21	13·87	0·80	80·56

TABLE VI

Chemical analyses of juice of Co 210, Co 213, Co 299, Co 313, Co 331 in 1936

No. of sample	Pests	Brix corrected	Percentage sucrose	Percentage glucose	Percentage purity
1	Co 331 Free from pest . .	18·74	15·52	0·80	82·81
2	,, Root-borer . .	18·74	15·57	0·93	83·07
3	,, Stem-borer . .	16·73	13·02	1·17	77·80
4	,, Top-borer . .	16·96	13·56	1·22	79·97
5	,, Root- and stem-borers	15·93	11·89	1·41	74·60
6	,, Root- and top-borers	12·78	9·11	1·19	71·32
7	,, Stem- and top-borers	13·42	9·59	1·45	71·42
8	,, Root-, stem- and top-borers	13·29	9·44	1·39	71·04
9	Co 213 Free from pest .	15·77	12·23	1·28	77·58
10	,, Root-borer . .	15·13	11·53	1·31	76·21
11	,, Stem-borer . .	13·88	9·81	1·54	70·70
12	,, Top-borer . .	13·68	10·17	1·21	72·32
13	,, Root- and stem-borers	15·49	11·77	1·34	75·91
14	,, Root- and top-borers	11·77	7·88	1·59	66·97
15	,, Stem- and top-borers	12·34	8·24	1·59	66·75
16	,, Root-, stem- and top-borers	11·11	6·66	1·64	59·92
17	Co 313 Free from pest .	21·11	18·05	0·49	85·51
18	,, Root-borer . .	22·08	19·00	0·45	86·05
19	,, Stem-borer . .	20·58	17·42	0·52	85·03
20	,, Top-borer . .	18·67	15·62	0·69	83·66
21	,, Root- and stem-borers	19·75	16·61	0·69	84·09
22	,, Root- and top-borers	19·85	16·70	0·57	84·12
23	,, Stem- and top-borers	17·13	13·31	1·00	77·70
24	,, Root-, stem- and top-borers	17·30	13·71	0·88	79·42
25	Co 210 Free from pest .	18·14	13·40	1·64	73·88
26	,, Root-borer . .	17·23	12·30	1·74	71·55
27	,, Stem-borer . .	17·60	12·92	1·64	73·41
28	,, Top-borer . .	17·74	12·91	1·75	72·79
29	,, Root- and stem-borers	17·68	12·65	1·86	71·54
30	,, Root- and top-borers	16·33	11·11	2·25	68·02
31	,, Stem- and top-borers	16·92	11·67	2·00	68·94
32	,, Root-, stem- and top-borers	15·17	9·7	2·17	63·06
33	Co 299 Free from pest .	20·78	17·02	0·99	81·89
34	,, Root-borer . .	21·28	17·61	1·08	82·76
35	,, Stem-borer . .	21·15	16·83	1·17	79·58
36	,, Top-borer . .	20·55	16·55	1·11	80·55
37	,, Root- and stem-borers	20·45	16·42	1·06	80·27
38	,, Root- and top-borers	19·05	14·82	1·39	77·81
39	,, Stem- and top-borers	19·95	15·99	1·20	80·17
40	,, Root-, stem- and top-borers	16·50	12·40	1·56	75·26

In 1936 the damage caused to the juice of Co 331 by the three borers was comparatively of the same order as in the previous year. Canes infested by stem, top and root-borers had 13·02 per cent, 13·56 per cent and 15·57 per cent sucrose content respectively, against 15·52 of the healthy ones. The stem-borer was most harmful, top-borer come next and the canes infested by root-borer had slightly higher sucrose content than even the healthy canes. It will, however, be noticed that the differences between the losses due to various borers were not so large as in 1935. Co 213 suffered due to stem and top-borers in the same way as Co 331, the canes infested by these borers having 9·81 per cent and 10·17 per cent sucrose content against 12·23 per cent of the healthy canes. But no increase in sucrose content was noticed due to root-borer.

In Co 313 and Co 299 top-borer caused more loss in sucrose than stem-borer, whereas in Co 210 the loss was more or less equal. The root-borer behaved in the case of Co 313 and Co 299 in the same manner as it did in Co 331 in 1935 and 1936. In the case of Co 210, as was the case in Co 213 described above, the root-borer decreased the sucrose content like other borers.

Summary and conclusions

A large amount of data on the incidence of various cane-borers and termites and the loss in weight and sucrose content due to the various pests occurring alone or in combination with one another were collected at Pusa at the harvest time (March) of cane in 1935 and 1936. In the former year one variety (Co 331) only was examined, while in 1936 five varieties commonly grown in North Bihar (Co 331, Co 313, Co 299, Co 213 and Co 210), which had been planted in February 1935 in randomised blocks, were under investigation. The results of the observations are briefly summarized below :—

A. Co 331 in 1935

1. About 78 per cent of canes were found infested by one pest or another, $22 \cdot 47 \pm 0 \cdot 89$ per cent being healthy (entirely free from pests). Top-borer had infested the highest percentage (about 20 per cent), root-borer was next in abundance (about 12 per cent), and stem-borer was found in the least number of canes (about 8 per cent). The number of canes attacked by termites was too small to lead to any reliable conclusions.

2. Regarding comparative loss of weight due to various pests, if healthy canes weighed 100 lbs., those infested by stem- and top-borers weighed about 68 and 95 lbs. respectively. Thus stem-borer caused the heaviest loss ($31 \cdot 96 \pm 3 \cdot 05$ per cent) and the loss due to top-borer ($5 \cdot 38 \pm 3 \cdot 20$ per cent) was insignificant. The canes infested by root-borer weighed more than even the healthy canes by about 24 per cent.

3. Regarding the actual loss in weight caused by various borers when occurring alone, with the extent of infestation mentioned in paragraph 1 above, the stem-borer was responsible for $2 \cdot 56 \pm 0 \cdot 33$ per cent and top-borer for $1 \cdot 02 \pm 9 \cdot 61$ per cent (not significant). The canes infested by root-borer showed an increase in weight by $2 \cdot 87 \pm 0 \cdot 24$ per cent.

4. The total loss in weight due to all pests combined was $5 \cdot 77 \pm 1 \cdot 71$ per cent (significant).

5. The canes infested by various pests showed deterioration in the quality of juice in the same order as in the case of weight described above. The stem-borer proved most harmful, the juice of canes attacked by this borer having 13·07 per cent sucrose against 16·2 per cent of the healthy canes. The top-borer-infested canes had 15·50 per cent, whereas the root-borer category had 17·37 per cent, about one per cent more than healthy canes. The purity of canes was also affected in the same order.

B. Co 331, Co 313, Co 299, Co 213, Co 210, in 1936

6. About 60 per cent of canes of Co 331 and Co 299, 70 per cent of Co 213, and 80 per cent of Co 313 and Co 210 were found infested by one pest or the other. The varietal differences were significant. As in the previous year in case of Co 331, top-borer was most common on all the varieties (about 13-24 per cent), root-borer came next (about 10-17 per cent), and stem-borer was the least abundant (about 6-14 per cent). The attack of various borers and varietal differences in this respect were both significant in the case of all varieties. The attack of termites alone or in combination with various borers was significant generally in the case of Co 210 only.

7. Whereas in 1935 the comparative loss in weight of Co 331 due to stem-borer was the heaviest, in 1936 the canes infested by top-borer showed the largest amount of loss in Co 331 as well as in all the other varieties examined, except Co 299, in which the loss was insignificant. The varietal differences were significant. The loss due to stem-borer was not significant in any variety. In Co 210 and Co 313 only loss due to root-borer was significant. In no variety (even Co 331) an increase in weight of cane infested by this borer was noticed, as was the case in Co 331 in 1935.

8. As regards the actual loss caused by the borers when occurring alone, with the extent of infestation summarized in para. 6 above, top-borer caused the heaviest loss in all varieties, varying between 0·67 and 5·45 per cent.

9. The total loss in weight due to all pests combined was significant in all varieties except Co 299. Co 210 suffered the most ($12 \cdot 81 \pm 2 \cdot 22$ per cent), Co 313 came next ($11 \cdot 62 \pm 2 \cdot 22$ per cent), followed by Co 213 ($9 \cdot 10 \pm 2 \cdot 22$ per cent), Co 331 ($7 \cdot 90 \pm 2 \cdot 22$ per cent). Co 299 suffered the least—$3 \cdot 92 \pm 2 \cdot 22$ per cent (not significant).

10. Though the highest loss in weight was observed to be due to top-borer in all varieties and the loss due to stem-borer was not significant in any variety (paras. 7 and 8 above), the highest decrease in sucrose content in the case of Co 331 and Co 213 was observed to be due to the latter borer. In

Co 313 and Co 299, however, top-borer caused more decrease in sucrose content and Co 210 suffered equally from the two borers.

As in the case of Co 331 in 1935, the juice of canes of Co 331, Co 313 and Co 299 infested by root-borer had higher sucrose content than even the healthy canes, though no increase in the weight of canes had been observed (paras. 7 and 8 above).

11. To summarize the results of two years, the top-borer was the commonest borer at Pusa on all the varieties, but the stem-borer seemed to be comparatively more harmful, especially in decreasing the sucrose content of the infested canes. The behaviour of the root-borer was remarkable. In 1935, in spite of its fairly high infestation, the canes of Co 331 attacked by it weighed more and had comparatively higher sucrose content than even the healthy ones. In 1936 though the infestation was almost as high as in the previous year, there was neither increase nor loss in weight. The sucrose content and purity of certain varieties (Co 331, Co 313, Co 299), however, was this year also comparatively slightly higher than that of the healthy canes.

It may therefore be concluded that the extent of infestation did not afford a reliable criterion of the actual loss caused by the pests. For instance in 1935, whereas top-borer was the most common on Co 331, the heaviest actual loss, both in weight and sucrose content, was caused by stem-borer. Further, the actual loss caused by the same pest to various varieties was not in proportion to its degree of incidence. For example Co 210 and Co 299 were found almost equally infested by top-borer in 1936, but whereas the former variety suffered the heaviest loss in weight, there was practically no loss to Co 299. Again Co 331 having less infestation (13·92 per cent) than Co 213 (15·43 per cent) suffered more loss (3·04 per cent) than the latter (2·49 per cent).

As regards the comparative behaviour of the five varieties examined in reference to all the pests considered together, Co 299 proved to be the best variety, as it had the lowest total infestation and suffered no significant loss at all. Co 210 proved to be the worst. The five varieties with regard to the actual total loss in weight sustained by them due to all pests combined could be arranged thus: Co 299< Co 331<Co 213<Co 313<Co 210.

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We take this opportunity of thanking Mr. P. V. Krishna Ayyar, M.A., Statistician, Imperial Agricultural Research Institute, for the trouble and interest he took in the analysis of our data. He has also gone through the manuscript and made some useful suggestions in reference to statistical interpretations of our observations. To the Imperial Agricultural Chemist we are indebted for the chemical analysis of the juice samples.

REFERENCE

Haldane, J. H. (1937). *Agric. & Live-stock in Ind.* **7**, 599-618

Appendix I

Variety Co 331

Number and weight (in pounds) of canes (eight replications) infested by various pests separately or in combination with one another in 1935

Replications / Combinations	1		2		3		4		5		6		7		8	
	No.	Weight in lbs.	No.	Weight in lbs.	No.	Weight in lbs.	No.	Weight in lbs.	No.	Weight in lbs.	No.	Weight in lbs.	No.	Weight in lbs.	No.	Weight in lbs.
Healthy	178	305·20	211	348	179	308·50	209	400	180	287	189	289·75	165	272·50	174	277·30
Root-borer	89	180·50	97	194·75	116	239·25	124	278	118	248·25	81	169·5	102	212·00	97	182·25
Stem-borer	54	69·25	62	61·25	93	91·25	54	63·25	90	104·13	66	67·50	59	67·80	51	67·18
Top-borer	136	222·63	153	228·8	136	216·5	154	234·25	173	301·75	198	278	198	306·5	179	297·50
Root- and stem-borers	18	38·05	14	21·50	17	31·75	20	34·13	6	11·63	10	21·5	6	12·95	6	9·25
Root- and top-borers	187	256·38	228	308·25	282	404·5	244	355	285	426·25	260	351·25	243	352·95	187	301·88
Stem- and top-borers	26	40·25	15	17·25	26	28·75	11	14	21	27	4	3·95	28	32·50	20	29·50
Root-, stem- and top-borers	24	38·95	22	25·05	46	64·50	23	36	31	40·75	19	24·50	33	42·00	43	63·00
Termites	2	2·25	4	5	5	6·25	1	2·38	2	4	4	8·75	...	...	...	...
Termites and root-borers	...	...	2	3·75	4	7·60	3	6	...	...	...	...	...	...	1	2·0
Termites and stem-borers	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Termites and top-borers	5	5·25	4	5	3	2·50	1	1·25	...	...	3	2·75	3	2·75	5	6·25
Termites, root- and stem-borers	...	...	...	...	1	1·80	...	...	...	...	...	...	...	...	...	...
Termites, root- and top-borers	...	...	1	1·25	9	10·25	4	5·75	...	...	...	...	...	...	...	...
Termites, stem- and top-borers	3	3	...	...	...	...	...	...	...	...	...	...	...	...	1	0·75
Termites, root-, stem- and top-borers	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

Appendix II

A. Variety Co 331

Number and weight (in pounds) of canes (eight replications) infested by various pests separately or in combination with one another in 1936

Replications / Combinations	1		2		3		4		5		6		7		8	
	No.	Weight in lbs.	No.	Weight in lbs.	No.	Weight in lbs.	No.	Weight in lbs.	No.	Weight in lbs.	No.	Weight in lbs.	No.	Weight in lbs.	No.	Weight in lbs.
Healthy	86	181	117	299	102	267	87	210·5	111	268·5	100	247	107	218	105	253
Root-borer	44	89·5	23	47	41	89·5	34	76·5	41	77·5	51	148	51	139·5	58	130
Stem-borer	25	59	24	50·5	27	58·5	9	22·5	24	45·5	36	67·5	23	52	21	51
Top-borer	23	38·5	38	60	32	71·5	61	123	40	84·5	23	42·5	27	50	35	53
Root- and stem-borers	10	21	7	16·5	11	26·5	10	20·5	17	35·5	19	37·5	13	27·5	9	16·5
Root- and top-borers	13	21·5	10	17	18	32·5	28	52	9	16	18	27	34	65	29	47
Stem- and top-borers	15	29	7	11	5	10·5	5	9	3	3·5	6	12	7	9·5	7	13·5
Root-, stem- and top-borers	3	7	3	3·5	3	7	4	7·5	3	1·5	4	9·5	6	15	...	...
Termites	3	6	...	...	7	11	...	...	5	10	1	3	2	4	1	2·5
Termites and root-borer	3	4	1	1	4	7·5	5	8·5	2	4	2	3	3	4·5	2	3
Termites and stem-borer	1	1·5	2	5	1	2·5	...	...	2	4	...	...	...	...	...	...
Termites and top-borer	...	...	...	...	1	2·5	...	...	...	...	...	...	1	2	...	...
Termites, root- and stem-borers	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Termites, root- and top-borers	...	...	...	...	...	...	2	5	...	...	...	...	...	...	...	...
Termites, stem- and top-borers	...	...	...	...	...	...	2	4·5	...	...	...	...	...	...	...	...
Termites, root-, stem- and top-borers	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

B. Variety Co 313

Number and weight (in pounds) of canes (eight replications) infested by various pests separately or in combination with one another in 1936

Replications / Combinations	1		2		3		4		5		6		7		8	
	No.	Weight in lbs.	No.	Weight in lbs.	No.	Weight in lbs.	No.	Weight in lbs.	No.	Weight in lbs.	No.	Weight in lbs.	No.	Weight in lbs.	No.	Weight in lbs.
Healthy	52	107·25	54	97	44	87	57	120	27	50·0	39	75·5	61	102	68	128
Root-borer	41	70	37	67	30	48	51	86·5	20	32·25	27	43·5	47	74·5	44	72
Stem-borer	26	50·5	22	45	35	62	25	45	37	66·5	45	82	36	57	54	86
Top-borer	46	86·5	43	67·5	43	64·5	24	40·5	24	38·5	30	46	35	52	36	54
Root- and stem-borers	30	44	25	39	29	52·5	24	41·5	27	57·25	33	53·5	28	52	42	65·5
Root- and top-borers	16	26·5	38	58	29	39·5	50	63·5	17	25·5	20	33·5	32	44·5	31	44·5
Stem- and top-borers	37	59·25	13	27·5	16	28	11	14·5	21	36	13	22	16	23	23	30·5
Root-, stem- and top-borers	16	25·5	22	34	26	38	18	23·5	43	62	15	21	17	29	21	29
Termites	1	1·25	...	...	3	5	...	...	...	...	1	2	5	11	...	...
Termites and root-borer	...	...	6	10	7	9	1	2	1	2·125	2	2·5	3	6	2	3
Termites and stem-borer	...	...	...	...	2	3·5	1	2	...	...	4	9	3	5	2	2·5
Termites and top-borer	...	...	...	...	1	2·5	1	2·5	...	...	1	1·5	1	1·5	1	2·5
Termites, root- and stem-borers	2	3·5	2	5	3	5	...	...	...	...	...	...	3	5	...	...
Termites, root- and top-borers	3	5·0	...	...	...	...	...	...	...	...	1	2	...	...	1	2
Termites, stem- and top-borers	...	...	...	...	...	...	1	1·75	...	...	2	5	...	...	2	2·5
Termites, root-, stem- and top-borers	2	3·5	4	6	1	3	...	...	...	...	1	1·5	...	...	...	...

C. VARIETY Co 299

Number and weight (in pounds) of canes (eight replications) infested by various pests separately or in combination with one another in 1936

Replications / Combinations	1		2		3		4		5		6		7		8	
	No.	Weight in lbs.	No.	Weight in lbs.	No.	Weight in lbs.	No.	Weight in lbs.	No.	Weight in lbs.	No.	Weight in lbs.	No.	Weight in lbs.	No.	Weight in lbs.
Healthy	128	179·5	94	129	161	210·5	122	158	138	176·5	184	220	152	210·5	159	221·5
Root-borer	40	55	34	50	34	38·5	38	42·5	43	55	64	68	67	67·5	64	81
Stem-borer	28	32·5	14	27	23	30	13	16·5	20	24	17	23	25	32	21	27·5
Top-borer	56	71	96	130·5	96	130	67	82	84	103	81	108·5	89	108·5	73	92
Root- and stem-borers	6	8	12	17	4	5·5	5	7	10	11	3	4	7	8	6	8·5
Root- and top-borers	40	50·5	40	47·5	35	33·5	25	27	35	38·5	8	6·5	30	35	30	35·5
Stem- and top-borers	8	12	13	16·5	17	23·5	18	18·5	14	20	15	14·5	10	13	6	8·5
Root-, stem- and top-borers	3	4	12	16·5	7	9	2	2·5	4	5	3	3·5	4	4·5	9	11
Termites	...	...	1	0·5	...	...	2	3	...	...	...	...	2	1·5	2	2
Termites and root-borer	1	1·25	1	1·0	...	...	...	...	1	0·5	...	...	...	...	...	...
Termites and stem-borer	1	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Termites and top-borer	...	...	...	...	...	...	1	1·75	...	...	...	...	1	0·5	...	...
Termites, root- and stem-borers	...	...	...	...	...	...	...	...	...	...	...	...	1	1·5	2	3
Termites, root- and top-borers	2	3	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Termites, stem- and top-borers	1	1·5	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Termites, root-, stem- and top borers	...	...	...	...	...	...	...	...	1	0·5	...	...	...	...	...	...

D. Variety Co 213

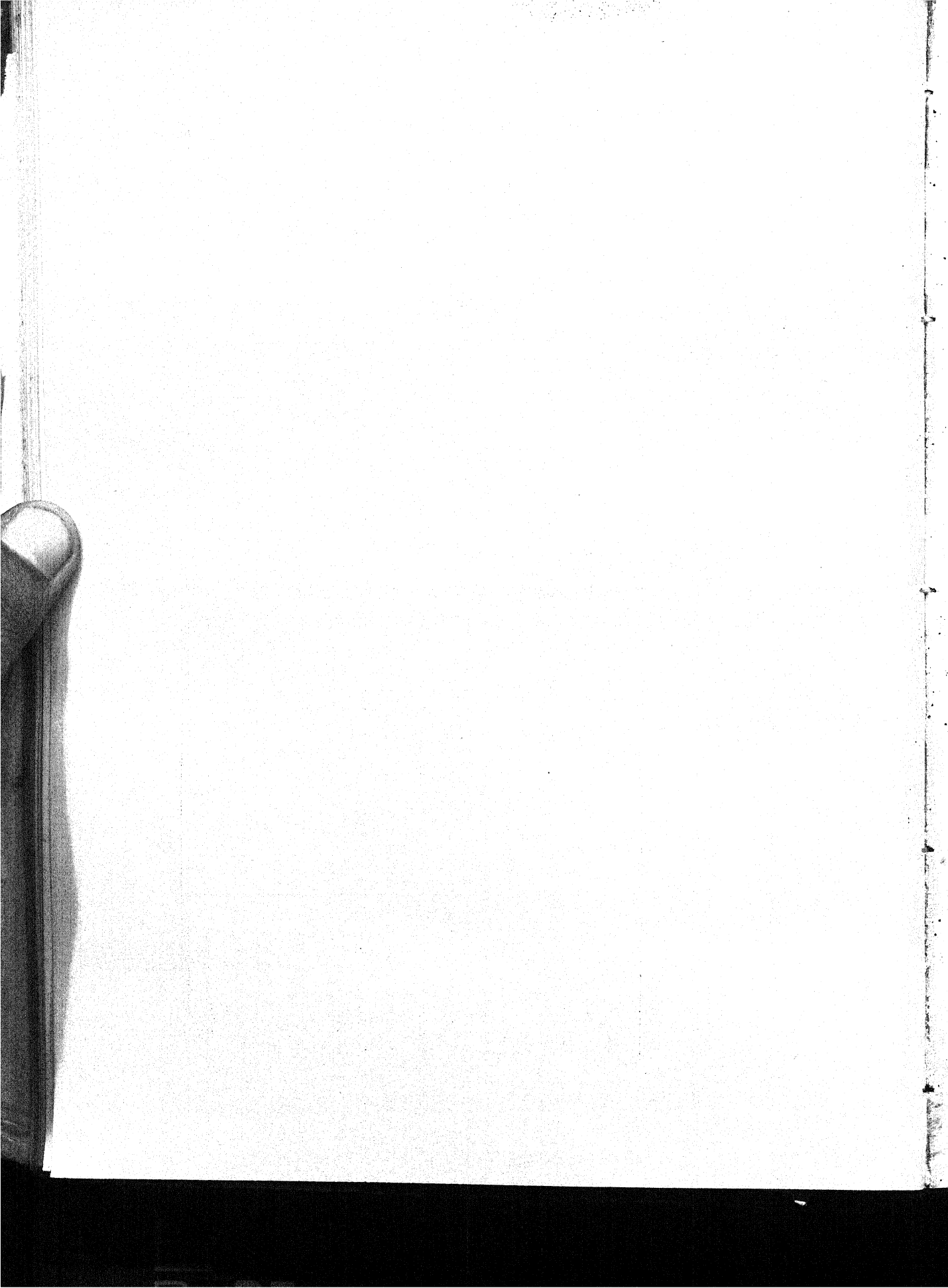
Number and weight (in pounds) of canes (eight replications) infested by various pests separately or in combination with one another in 1936

Replications / Combinations	1		2		3		4		5		6		7		8	
	No.	Weight in lbs.	No.	Weight in lbs.	No.	Weight in lbs.	No.	Weight in lbs.	No.	Weight in lbs.	No.	Weight in lbs.	No.	Weight in lbs.	No.	Weight in lbs.
Healthy	56	110	100	149	149	270·5	154	238	108	202	114	206·5	108	196·5	87	131
Root-borer	23	37·5	28	43·5	45	73	28	46	29	48	34	62	36	97·5	30	40
Stem-borer	55	100	44	76	28	47·5	86	159	63	112·5	44	75·5	31	78·5	34	56
Top-borer	52	87	55	86·5	46	69·5	59	64·5	42	59	56	82·5	66	98·5	50	63
Root- and stem-borers	19	26	18	31·5	16	24·5	20	31·5	10	20	26	47	23	34·5	19	29
Root- and top-borers	20	30	13	14·5	18	24·5	22	28·5	6	9·5	24	34·5	35	47·5	37	41·5
Stem- and top-borers	48	82·5	22	31·5	14	20	43	62	12	12·5	33	44	11	16	30	38·5
Root-, stem- and top-borers	18	23	20	28	4	6	20	21·5	2	2·5	13	15	12	17	33	35·5
Termites	...	...	3	5	1	1	...	...	7	13	...	...	2	3·5	2	3·5
Termites and root-borer	1	1·8	10	16·5	9	13·5	4	3	5	8	4	8	5	8·5	7	10
Termites and stem-borer	3	4	4	5	3	3	...	...	3	4·5	...	...	...	...	...	...
Termites and top-borer	3	4·5	2	3	...	...	...	...	2	3	1	1·5	...	...	...	...
Termites, root- and stem-borers	...	...	4	7	...	...	1	2·5	2	3	5	8·5	3	4·5	5	7
Termites, root- and top-borers	...	...	1	1·4	6	9·5	...	...	...	...	8	13·5	12	14	3	3
Termites, stem- and top-borers	3	5	3	5·5	...	...	...	...	...	...	...	...	...	...	...	...
Termites, root-, stem- and top-borers	1	1·5	...	...	1	1·25	1	0·75	1	1·5	1	2	6	7·5	2	3·5

E. Variety Co 210

Number and weight (in pounds) of canes (eight replications) infested by various pests separately or in combination with one another in 1936

Replications / Combinations	1		2		3		4		5		6		7		8	
	No.	Weight in lbs.	No.	Weight in lbs.	No.	Weight in lbs.	No.	Weight in lbs.	No.	Weight in lbs.	No.	Weight in lbs.	No.	Weight in lbs.	No.	Weight in lbs.
Healthy	79	126	63	105	55	89	66	125	86	160·5	90	161·5	117	215	58	104
Root-borer	36	47	40	60·5	40	63	46	69	40	78	34	52	34	60	32	48
Stem-borer	45	70	22	35	26	47	18	27	40	70	35	55·5	33	58	10	16
Top-borer	63	88	81	123	77	105	85	117·5	61	86	108	149	80	133	96	127·5
Root- and stem-borers	18	28·5	4	8	10	14	10	15	23	32	6	8·5	15	20·5	10	19
Root- and top-borers	42	56	55	86	47	66	57	77	19	27	32	41·5	26	38	97	124
Stem- and top-borers	20	26	9	17	25	36	25	33	26	41·5	23	24	22	38	18	24·5
Root-, stem- and top-borers	21	26·5	3	5	15	21	17	22	9	12	4	6	23	31	10	11·5
Termites	4	4·5	8	11	8	10	11	23	...	...	...	...	1	2	...	...
Termites and root-borer	5	6·5	3	5	9	10	8	9·5	3	5	2	3·5	7	10·5	1	1·5
Termites and stem-borer	3	5·5	...	...	3	4·5	5	8	5	6·5	...	...	...	...	...	...
Termites and top-borer	4	6	5	8	8	10	3	4	2	3	...	...	1	1·5	4	5·5
Termites, root- and stem-borers	...	...	...	...	2	4·5	...	...	...	...	2	3·5	3	4	3	4
Termites, root- and top-borers	...	...	...	...	5	6	5	8·5	3	4·5	7	8	13	17	10	10
Termites, stem- and top-borers	...	...	3	4	1	2	3	3·5	1	1·5	...	...	...	...	...	...
Termites, root-, stem- and top-borers	2	3	...	...	6	7	3	4	1	1·5	2	3	6	6·5	7	8·5



THE PROTEINS IN THE RICE GRAIN AND THE DEVELOPMENT OF THE ALEURONE LAYER

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(With Plates II—IV)

I. Introduction

In connection with the investigation on 'quality in rice' financed by the Imperial Council of Agricultural Research, histological studies of the grain were undertaken at Coimbatore. Some results dealing with the formation of starch, and different colours of the pericarp in the developing grain have already been published [Rajasekhara Mudaliar, 1936 and Ramiah *et al.*, 1937]. The next step in the investigations was (1) to study the nature of the proteins occurring in the aleurone and endosperm cells of the developing grain by micro-chemical methods and (2) to study histologically the differences, if any, in the thickness of the aleurone layer in some typical rices and to determine how far the environmental conditions influence this layer. Since the aleurone layer is known to be the principal seat of rice proteins and since the nutritive value of rice is essentially related to its protein contents besides minerals, the differences in the thickness of the aleurone layer should give a comparative value of the rice quality. The present paper deals with results obtained in these two investigations.

II. Formation of proteins

The existence of aleurone layer in cereals and the large concentration of proteins in it have been recognised by several botanists [Hartig, 1855; Maschke, 1859; Sachs, 1862 and Pfeffer, 1879]; Schenk [1872] [quoted by O'Brein, 1895] found in wheat that Millon's reagent did not affect the aleurone layer, and on this ground he concluded that aleurone was of low nutritive value. He, however, found that the portion of the endosperm adhering to the aleurone was rich in nutritive value. Groom [1893] found that aleurone grains in Gramineae contained double phosphate of calcium and magnesium characteristic of globoids. O'Brein [1895], examining aleurone layers in wheat, stated (1) that there was continuity between the aleurone layer and the endosperm

by protoplasmic network; (2) that the cells adjacent to the aleurone layer were specially rich in protein matter (gluten); (3) that the aleurone layer came into close relationship with the embryo by the continuation of its cells over the external surface of the edge of the scutellum; (4) that the protoplasm of the embryo was continuous with that of the adjoining aleurone cells and the underlying endosperm and (5) that much oil was present in the aleurone cells and perhaps some carbohydrate also. He suggested that the aleurone functioned as part of the store for the embryo and also helped in conducting food materials.

To study the nature of the proteins occurring in the aleurone and the endosperm cells, micro-chemical studies were undertaken. Very little work has been done hitherto on the micro-chemical study of the proteins in rice grain. Dastur [1935] has made a study of proteins in the rice grain, but the substance orthoquinone, one of the constituents recommended for his reagent, could not, however, be obtained anywhere. Considerable attention had therefore to be devoted first to determine the most satisfactory reagent to give the best stain reaction.

Micro-chemical studies

After testing several reagents, Millon's and Adamkiewicz's reagents were found to give best results, and these have been used in the present investigation. With Millon's reagent, proteins were stained red in all parts of the grain, but this reagent did not give a reaction with aleurone grains in materials tested seven days after fertilisation. Adamkiewicz's reagent gave characteristic reddish violet colour to proteins in the aleurone layers in the same material. Hopkin's and Cole's modification of Adamkiewicz's reagent and syrupy phosphoric acid also gave fairly good results but not quite so definite as Adamkiewicz's. Takahashi [1913], working on proteins of rice, found that all four kinds of proteins occurring in rice—albumin, globulin, prolamin and glutelin—gave positive reactions with Millon's, Adamkiewicz's and Biuret reagents. But as has already been observed in regard to histological sections the first two reagents only were found to be satisfactory.

Though rice is supposed to contain a fairly large proportion of glutelin [Allen, 1930], which is insoluble in water and alcohol, the stain reaction could not be obtained in materials fixed in different fixatives and passed through all stages of dehydration, hardening, etc. Therefore fresh specimens only were used throughout, and free-hand sections were taken for observing the stain reactions. The exact position of spikelets on the panicle was first marked on a paper just when the ear-head had emerged from the sheath, and as the flower opened every day, the date of flowering was marked against each individual spikelet. The exact day of anthesis having thus been recorded for each spikelet, it was found easy to collect ovaries of particular age for free-hand sectioning and staining. Formation of protein was observed from the third day after anthesis right up to the maturity of the grain. Ripe grains had to be soaked several hours in water before taking sections.

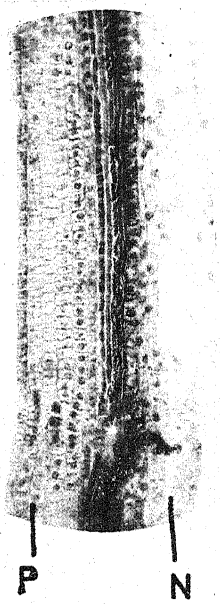

FIG. 1. Longitudinal section of rice ovule 3 days old, showing the ovary wall and a portion of nucellus. Periphery of nucellus richer in proteins
P—Pericarp ; (ovary wall)
N—Nucellus

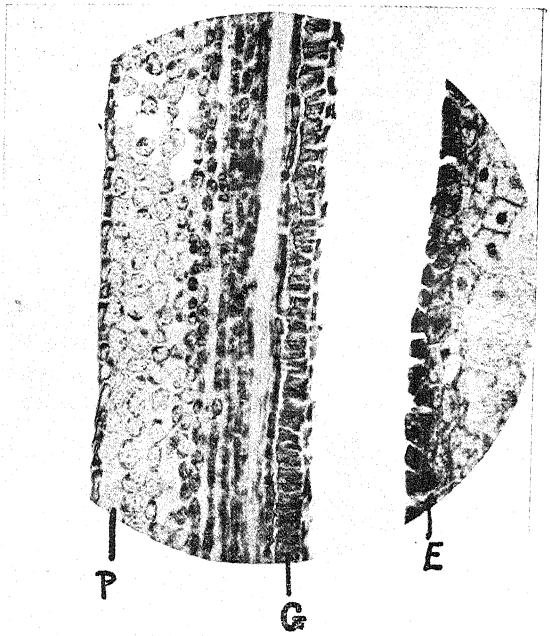

FIG. 2. Longitudinal section of rice grain 5 days old showing ovary wall and a portion of endosperm. Aleurone layers are not yet differentiated. Peripheral cells of the endosperm take deeper stain, suggesting more of proteins
G—Epidermis of nucellus
E—Endosperm

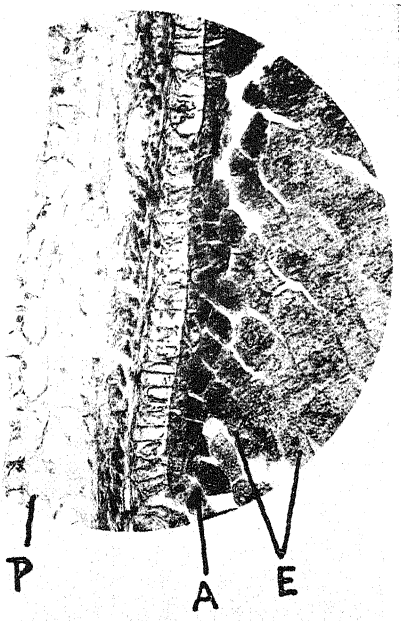

FIG. 3. Longitudinal section of rice grain 7 days old. Aleurone layers are clearly differentiated and are not affected by Millon's reagent but stained by Adamkiewicz's reagent.
A—Aleurone ;
E—Peripheral cells of endosperm which take deeper stain with Millon's reagent.

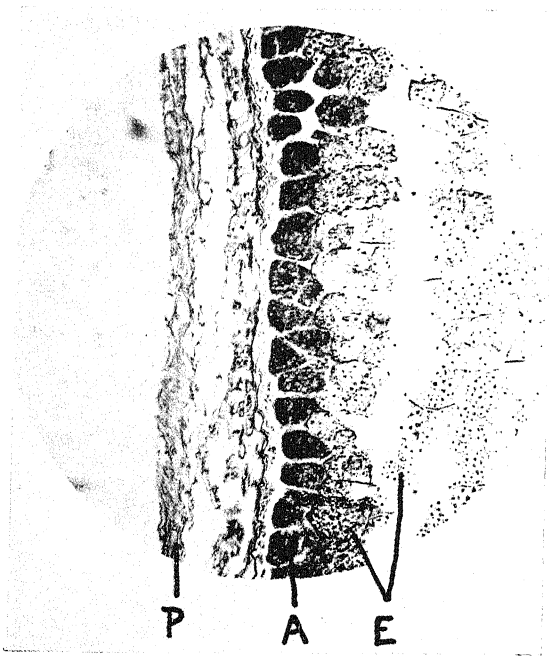

FIG. 4. Longitudinal section of rice grain 30 days old. Pericarp very much reduced. Aleurone layer, A, is very prominent. Stain reaction same as for Fig. 3.

Observations

The following observations were recorded—

Third day after anthesis.—The cells of the ovary wall take a slight reddish colour with Millon's reagent. The conducting strands and the innermost layer of cells of the ovary wall take a deeper stain suggesting larger amounts of proteins. The nucellus was also stained light red but the colour was more towards the periphery than towards the centre of the grain (Plate II, fig. 1).

Fourth and fifth days after anthesis.—With rapid development of the endosperm tissue, the proteins in the ovary wall get gradually exhausted. The outermost layer of cells of the endosperm (aleurone) take a deeper stain than the remaining portion (Plate II, fig. 2).

Sixth day after anthesis.—The aleurone is not clearly differentiated from the endosperm tissue, but the outermost three or four layers take a dark red stain and the colour fades away gradually towards the centre. At this stage the ovary wall contains very little protein.

Seventh day after anthesis.—On the seventh day and thereafter up to the maturity of the grain, the outermost layers of cells of the endosperm were not stained by Millon's reagent while one or two layers adjacent to the aleurone showed an intense colour reaction to the stain. Adamkiewicz's reagent, however, gave the characteristic reddish violet stain in the outermost layers which did not react to Millon's reagent (Plate II, figs. 3 and 4).

Discussion of results

The action of Millon's reagent on the aleurone of rice grains seven days after anthesis corresponds with the observations recorded by Schenk [1872] in wheat, but the action of Adamkiewicz's reagent definitely proves the presence of proteins in the aleurone. It is possible that the occurrence of some other substance in the aleurone after this stage retards the action of Millon's reagent. Lloyd [1926] states that the colour tests for proteins are not due to proteins as such but due to certain groups (amino-acids) commonly occurring in them, that tryptophane is responsible for the colour reaction obtained with Adamkiewicz's reagent, and that tyrosine brings about the colour changes due to Millon's reagent. It is probable that the aleurone layer in materials tested seven days after fertilisation consists mostly of tryptophane, while the few layers of the endosperm adjoining the aleurone consists mainly of tyrosine. Lloyd [1926] states that tryptophane has some individual role to fulfil in metabolism over and above that of supplying a quota of nitrogen to the system. Aykroyd and Hopkins [1916] showed that removal of tryptophane from the diet of young growing rats was immediately followed by a rapid loss of weight, and its restoration produced renewed growth. Tryptophane therefore appears to be an essential constituent of animal diet, and since the aleurone is rich in tryptophane it follows that this layer is the most important nutritive portion of rice. The seat of tyrosine in the rice grain may be the first one or two layers of cells

adjoining the aleurone layer. The evidences of the importance of tyrosine in the diet of animals are conflicting. In the case of rats, its presence was not found to be so very essential [Totani, 1916].

The occurrence of large quantities of proteins in the peripheral layers of the endosperm confirms O'Brein's [1895] observations in wheat and it is no doubt due to the protoplasmic network connecting the aleurone with the endosperm. O'Brein's findings about the protoplasmic connections between the embryo and the surrounding aleurone layer are also borne out in rice. It is observed in rice sections that portions of coleoptile, plumule, coleorhiza, and root tips which are adjacent to the aleurone layer take a deeper stain reaction with Adamkiewicz's reagent than the interior portions. It is clear that the proteins are first concentrated in aleurone layers and later (seven days and more after fertilization) they pass on to the adjacent endosperm cells and those parts of the embryo which are nearer to the aleurone layer. Similar observations have also been reported by Yamasaki [1933] in wheat; he states that there are one or two layers of starch cells of a special type adjoining the aleurone which have thicker cell walls and are rich in gluten content. Some preliminary observations of market samples of rices have revealed that in all samples examined, the aleurone layers have been removed completely, thereby indicating that tryptophane which is an important principle of diet is not obtainable in highly polished market rices. Even the less important constituent, tyrosine, is absent in several samples.

III. Histological studies

The only reference available regarding the histological study of the aleurone layer in rice grain is that of Iliffe [1929] who measured the thickness of the aleurone layer in some rice varieties at the Paddy Breeding Station, Coimbatore.

Material and method

To measure the thickness of the aleurone layer in different types of rices and in rices grown under different manurial treatments, a special method had to be devised. A few varieties in each of the following groups were studied.

1. White rice varieties of different duration.
2. White and coloured rices.
3. Soft, hard, glutinous and wild rices.
4. Rices from different manurial experiments.

The method of taking specimens of required age and fixing, cutting, staining, etc., are explained by Rajasekhara Mudaliar [1936]. This method is slightly different from the one adopted by Brenchley [1909] for cutting sections of wheat grain. In this method, ovaries of above fifteen days' age were subjected to special treatment. After fixing in formalin-acetic-alcohol the grains were treated with a mixture of one-third glycerine and two-thirds

take deeper stain with Millon's reagent.

VARIATIONS IN ALEURONE THICKNESS IN THE WHITE RICES

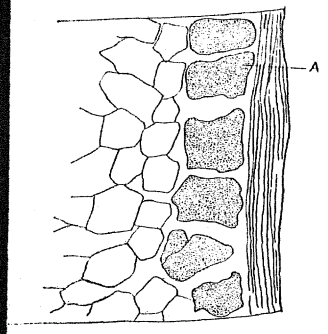

FIG. 1. GEB 24

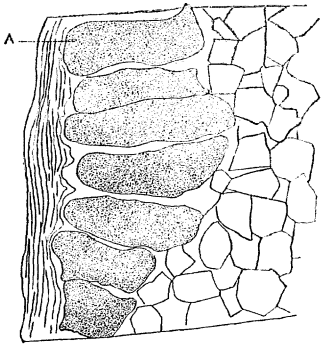

FIG. 2. Co 4

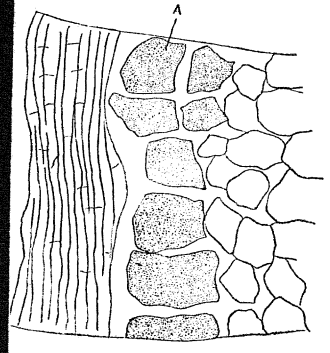

FIG. 3. Co 10

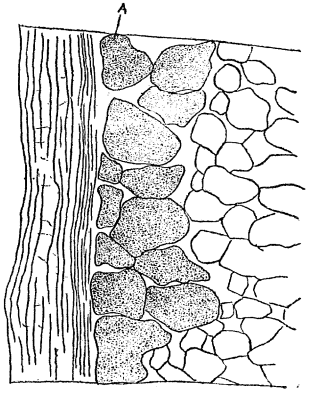

FIG. 4. Co 5

FIGS. 1–4. Sections of white rice varieties, drawn to same magnification (×300)
The good development of aleurone in the coarser variety, Co. 4 may be noticed
A—Aleurone

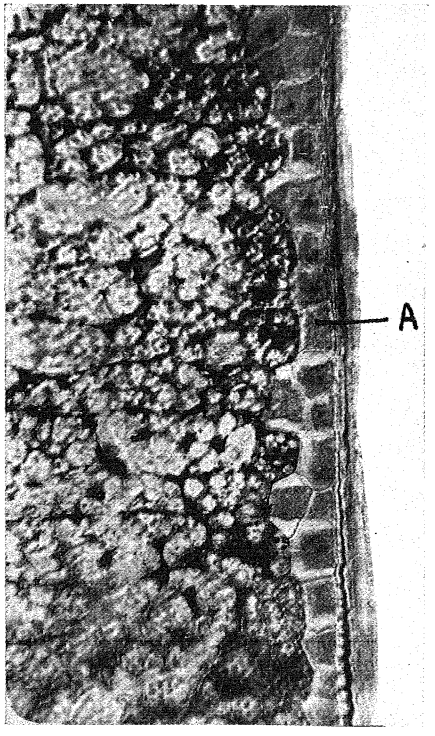

FIG. 5. Longitudinal section of the rice grain obtained with 4,000 lbs. green leaf
A—Aleurone on the dorsal side of the grain

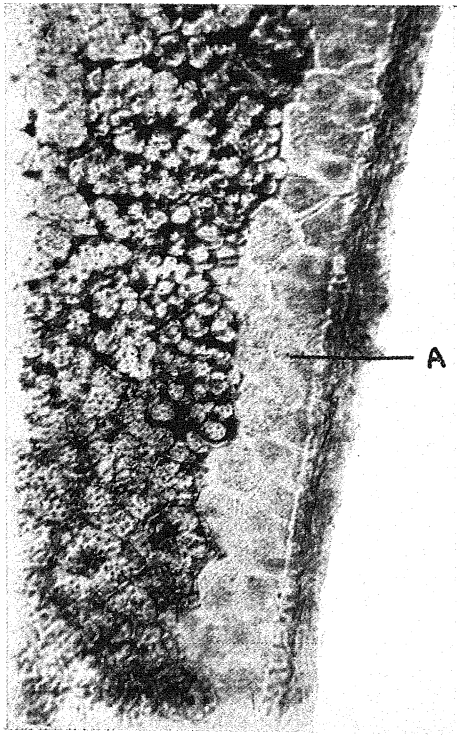

FIG. 6. Longitudinal section of the rice grain obtained by manuring the crop with 20,000 lbs. green leaf per acre. The extraordinary development of the aleurone in the manured seed may be noted

rectified spirit for about a week and then passed on to paraffin infiltration through the medium of Bergamot oil. The embedded material was cut and the cut surface was soaked in water for at least twelve hours before cutting the sections. Longitudinal sections, 10 to 12 μ thick, were then cut and stained in picro-anilin-blue. This stain gave to the aleurone layers a rich blue colour, while the endosperm took a light green stain. The growth and development of the aleurone layer were studied at three different stages of growth, namely, seven, sixteen and thirty days after fertilisation.

Some general observations on the formation and development of aleurone grains may also be recorded. The aleurone grains on the ventral side (the side along the embryo) are generally single layered, while those on the dorsal side (the side opposite to the embryo) are generally two to three celled. Even the individual cells of the aleurone are thicker on the dorsal side than on the ventral. The aleurone layers surrounding the embryo all round are very thin, even thinner than those on the ventral side, and they gradually increase in thickness towards the middle of the grain. This thickness is generally maintained up to the tip of the grain. The aleurone layers below the conducting strands, which traverse the grain on the dorsal side, are the thickest. Just seven days after anthesis, one or two peripheral layers of the endosperm get denser in cell contents, and take a deeper stain with picro-anilin-blue, and these cells ultimately differentiate as the aleurone layers. At this stage vacuolation is faintly seen in the aleurone cells, and it becomes prominent with the ripening of the grain. The cell contents of the aleurone get denser till the sixteenth day, after which they begin to contract and recede towards a side wall, and at the ripening of the grain they appear contracted in most cases. Measurements of aleurone grains were taken from both the dorsal and the ventral side and each measurement consisted of six to ten readings.

White rices of different duration.—Seven varieties were examined under this group of which one was of short duration, two of late duration and four of medium duration (Table I). The most striking difference noted between the short and long duration varieties is that even on the seventh day after anthesis, the aleurone is much better developed and differentiated in the short duration variety than in the long duration one. In the two varieties belonging to the latter group, Adt 11 and Co 4, the formation of the aleurone is not observed till the seventh day, whereas in varieties of medium duration, *e.g.*, GEB 24, Co 1, Co 5 and Co 7 it did appear on that day. As regards the final stages of development there are no apparent differences among different groups. Variation in the aleurone thickness among these rices can be seen from Plate III, figs. 1—4.

White and coloured rices.—During his preliminary studies on the histology of the rice grain, Iliffe [1929] has recorded that there are variations in thickness of the aleurone in differently coloured rices. The aleurone was slightly thicker in red rices than in white ones. A summary of results obtained by him and later published by Ramiah [1936] is given below in a tabular form.

Thickness of bran layers in rice

Variety	Grain size (coarse, fine, etc.)	Thickness of bran layer in microns (Pericarp+Aleurone)		Rice colour
		Dorsal side	Ventral side	
Black puttu	Coarse	62·3	40·5	Purple
T 303	Do.	53·3	37·4	White
T 326	Do.	54·1	32·8	Do.
T 88	Do.	48·8	32·1	Grey brown
T 219	Do.	50·1	30·6	White
Co 4	Do.	48·1	28·5	Do.
T 29	Do.	52·8	39·3	Red
T 248	Do.	44·1	38·3	Do.
T 375	Do.	41·0	31·3	Do.
T 73	Medium	54·8	33·8	Do.
T 132	Do.	56·6	39·1	Brown
Co 6	Do.	43·8	28·8	White
Co 5	Do.	44·5	30·3	Do.
Co 7	Do.	41·8	31·6	Do.
Co 2	Do.	47·8	28·8	Do.
T 218 (Adt 4)	Do.	36·8	21·3	Do.
T 217 (Adt 3)	Do.	36·0	22·0	Do.
Co 3	Do.	34·3	22·0	Do.
Co 1	Medium fine	42·3	25·8	Do.
T 2	Fine	42·6	25·6	Do.
T 124	Do.	47·1	27·3	Do.
T 109	Do.	37·8	24·8	Do.
GEB 24	Do.	35·3	26·1	Do.

WHITE AND COLOURED RICES

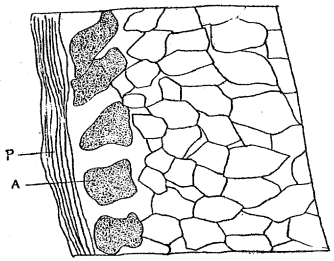

FIG. 1. White

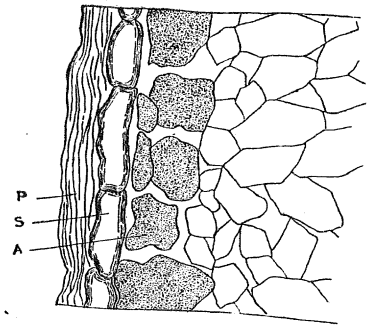

FIG. 2. Red

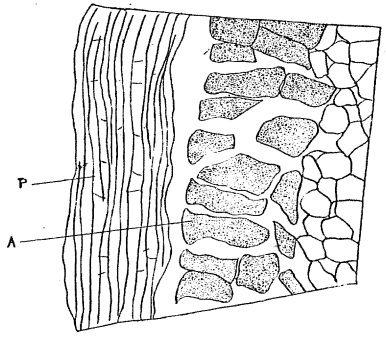

FIG. 3. Dark purple

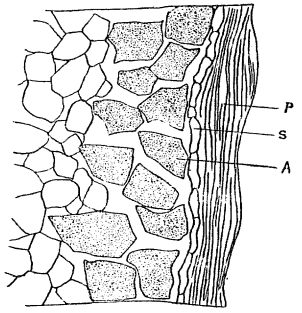

FIG. 4. Brown

FIGS. 1–4. Longitudinal sections of different coloured rices (×300) all drawn to same magnification

P—Pericarp

CULTIVATED AND WILD RICES

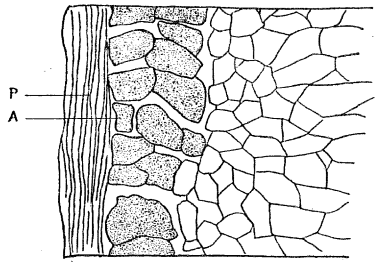

FIG. 5. Cultivated rice

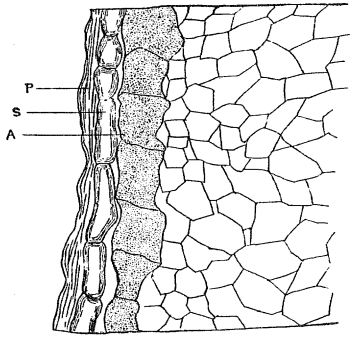

FIG. 6. Wild rice

FIGS. 5–6. Longitudinal sections of cultivated and wild rice. The poor development of aleurone in the wild rices may be noticed. (×300)

S—Spermoderm

To confirm these observations further, the thickness of aleurone was measured at three different stages, in eight different varieties, of which three are the same as those mentioned in Table I. The description of these varieties and the measurements are given in Table II. It will be seen that besides colour, the varieties differ in size also, some being definitely coarser than others.

It is generally observed that in coloured rices examined on the seventh day after anthesis, the aleurone can be seen on the dorsal side alone while on the ventral side it is not yet differentiated, the purple rice being an exception. In the grey-brown rice, the aleurone on the ventral side is not distinguishable even on the sixteenth day. In this rice, the aleurone development is poor and even at the ripening time, the cell contents are not concentrated and vacuolated like other varieties.

Glutinous, hard, soft and wild rices.—In this group five varieties—two glutinous (black and white), one non-glutinous but hard and translucent, one non-glutinous but soft and opaque and a wild rice (*O. officinalis*)—were examined (Table III). The glutinous varieties selected for the study show the greatest development of aleurone so far examined. The increase of aleurone thickness in them is more marked during seventh to sixteenth day.

Results.—The results given in Tables I to III may be briefly examined. It may be stated at the outset that in order to get differences in thickness of the aleurone layer between different groups of rices, such as early or late, white or red, coarse or fine, and glutinous or non-glutinous, a much larger number of varieties should have been examined under each group independently. It is only then that the data could lend themselves to a statistical analysis. As it is, the inferences drawn are only indicative. The results in Table I do not show any striking differences except that in an early variety, Co 10, the formation of aleurone is much earlier than in others. The small increase in aleurone thickness in the case of Co 7, Co 4 and Adt 11 might probably be due to their being comparatively coarser grained than others. From Table II, it will be seen that in red rices, T 111 and Co 9, the aleurone is thinner in the finer one (T 111) than in the coarser type, Co 9. This difference is, however, not quite so definite when two white rices, a fine and a coarse (GEB 24 and Co 4), are compared, but then it must be said that Co 4 is not quite so coarse in comparison with GEB 24 as Co 9 is with T 111. Among coloured rices differences do exist as are visible in Plate IV, figs. 1-4. Colour as such does not appear to influence the thickness of the aleurone layer but the indication that coarser rices, whether coloured or white, have thicker layers than finer rices appears to be more definite. Though previous workers [Kikkawa, 1919 and others] have recorded the existence of empty spaces or gaps in the structure of grains indicative of their softness, the soft rices examined have not revealed the presence of such empty spaces. Comparison of a hard (T 167) and a soft (T 523) rice definitely shows that the aleurone is thicker in the former than in the latter (Table III). In regard to wild rice (T 573) the aleurone layer appears thin when compared to cultivated rices (Plate IV, figs. 5 and 6). The micro-photographs clearly show

that the aleurone has not concentrated and receded from the cell wall even at ripening in many of the cultivated rices. As the pericarp colour is red, the colouring layer, spermoderm, is very conspicuous.

Effect of environment on aleurone development.—(*a*) The most important environmental factor that may normally be expected to have the most profound effect on the plant is the manure applied to the soil in which the plant is grown. In a manurial experiment conducted at four centres, *i.e.*, Coimbatore, Maruteru, Berhampur and Pattambi, the same variety of paddy, *i.e.*, GEB 24 was planted, and its grains were examined for aleurone thickness. The following four identical treatments were given at all centres :—

A.—No manure.

B.—Ammonium sulphate to supply 30 lb. nitrogen per acre.

C.—Sodium nitrate to supply 30 lb. nitrogen per acre.

D.—Green leaf to supply 30 lb. nitrogen per acre.

The measurements of aleurone thickness in grains thirty days after anthesis are recorded in Table IV. Measurements were taken only on the dorsal side. The results are definite and interesting. Taking A as control, the effect of treatment D in increasing the aleurone thickness is significant at all four centres. The effects of B and C have been variable in the different centres, but next to D, treatment C has got a significant effect at three centres, Maruteru, Pattambi and Berhampur. The difference between C and D is significant at Maruteru and Coimbatore and there is no difference among treatments A, B and C at Coimbatore.

The application of bulky organic manures, like green leaf, has contributed to the maximum thickness of the aleurone layer and next to green leaf comes the chemical fertiliser in the form of sodium nitrate. Taking aleurone thickness as an indication of grain quality, of the two chemical fertilisers, sodium nitrate and ammonium sulphate, the former contributes more to the quality of the produce while the latter to the quantity.

Apart from the effects of the manure applied, something might be said of the effect of the place also on the development of the aleurone. The Maruteru soils being clay are richest and ideal for paddy cultivation, while those of Pattambi poorest, being shallow laterite. The soils of Coimbatore and Berhampur are intermediate in character. Taking treatments A and D alone into consideration, the thickness of aleurone in A is lowest in Pattambi and highest in Maruteru and the increase in thickness due to treatment is significantly high in Maruteru compared to these treatments at other three centres. It must be stated, however, that the seed grown in three centres other than Coimbatore was not sent from Coimbatore during the year of the experiment but was the produce of a crop grown locally for some years with the seed originally obtained from Coimbatore. It may be suggested that the big difference shown at Maruteru might have been brought about by cumulative effect, since this station has been growing this variety for a number of years.

(*b*) Advantage was taken of another seed-bed manurial experiment in progress at Coimbatore to examine the produce for the thickness of the aleurone layer. The grain examined (Co 1) came from the two treatments mentioned below :—

A.—No manure in both seed-bed and transplant field.

B.—Heavily manured seed-bed (20,000 lb. green leaf+1 cwt. each of ammonium sulphate and super phosphate per acre) and normally manured transplant field (4,000 lb. green leaf+1 cwt. of ammonium sulphate).

The measurements of the grain are recorded in Table V. Unlike the previous experiment, aleurone thickness was measured at three stages of development both on the dorsal and ventral sides. The difference between the treatments at the first two stages is not significant but it is highly significant at the last stage, particularly on the dorsal side. The better development of the aleurone in treatment B is evident from the sections of grains (Plate III, figs. 5 and 6).

Discussion.—Iliffe [1929] suggested that coloured varieties generally have thicker aleurone layers. This has not been brought out definitely in the present investigation though there are some indications to that effect. The examination of a larger number of samples would settle the point. On the other hand, the fact that coarser varieties have thicker aleurone layers than finer ones, irrespective of colour is more definitely brought out. This agrees with the findings of the Bio-Chemist [Anonymous, 1937] in charge of the quality investigations in Bangalore, who as a result of chemical analysis and actual feeding trials with pigeons has stated that " coloured and coarse grained varieties of rice contain larger amounts of proteins and mineral matter than the finer ones ". This should mean that with the same amount of polishing the coloured and coarser rices will lose much less of nutrient matter than the finer ones. These findings support the general opinion prevalent even now in rural parts that coloured and big rices are comparatively more nutritious and labouring classes often state that such rices are more sustaining than the so-called superior finer rices. That glutinous rice is also more nutritious than the non-glutinous is confirmed by these investigations.

McCarrison [1924], as a result of his investigations on the nutritive values of rices in South India, considered that these might vary with soil conditions. Again he found (quoted by Viswanath) that the nutritive value of *ragi* (*Eleusine coracana*) varied with the manurial treatment of the soil. The present investigations of the effect of environmental conditions, such as the nature of soil and manuring, on the quality of rice grains as shown by the aleurone thickness confirm McCarrison's conclusions.

In fact experimental evidence has been produced to confirm the popular opinion commonly expressed that the same variety of rice will vary in nutritive quality according to the place in which it is grown. Regarding the effect of organic and chemical fertilisers on the quality of the crop, Viswanath *et al.* [1926] concluded (*i*) that a crop manured with organic manure (cattle

manure) gave seed with a better cropping value than that from a crop manured with mineral manure or not manured at all; (*ii*) that mineral manure was better than no manure from the point of view of the quality of the seed. These observations were confirmed by McCarrison who simultaneously carried out nutrition experiments with animals, with the same materials used by Viswanath for his studies. The present investigations have shown that organic manure (green manure) has the greatest effect in increasing the thickness of aleurone, followed by mineral manures, of the type of sodium nitrate, over the no-manure treatment. Nothing can be said, however, of the better cropping value of the produce from the field manured with organic manure. The increase in thickness of the aleurone naturally improves the nutritive quality of the grain, but it does not follow that such an improvement should mean an improvement in the cropping quality. The present investigation is the first recorded instance of manuring or soil conditions having a direct effect on the structure of the grain which is indirectly related to its nutritive value.

Summary of results

(*i*) Millon's and Adamkiewicz's reagents were used to trace the formation of proteins and the latter was found to be more satisfactory.

(*ii*) Small quantities of proteins are observed in the ovary wall up to the fifth or sixth day after anthesis and they get gradually exhausted after the development of the aleurone and starch cells.

(*iii*) The composition of proteins in the aleurone layer probably changes into some other form (seventh day after anthesis). It is quite likely that the aleurone after this stage consists mostly of tryptophane while proteins in other portions of the grain are mainly composed of tyrosine.

(*iv*) One or two layers of cells of the endosperm adjacent to the aleurone are rich in proteins. In the embryo, the concentration of proteins are more towards the portions adjacent to the aleurone layer.

(*v*) The aleurone cells on the ventral side of the grain are generally single layered, while on the dorsal they are two to three celled; these are thickest just below the conducting strands which traverse the grain on the dorsal side.

(*vi*) There are indications to show that in an early variety the aleurone develops earlier than in the late varieties.

(*vii*) There is a slight indication that coloured rices have thicker aleurone than the white rices, but the aleurone thickness seems to depend to a large extent on the coarseness of the grain. The aleurone development is at its maximum in the purple rice.

(*viii*) Maximum development of aleurone has been observed in the glutinous rices, while the poorest development is recorded in a wild rice.

(*ix*) Manurial experiments with green leaf and chemical fertilisers, like ammonium sulphate and sodium nitrate, conducted at four centres with the same varieties of rice, have shown that green leaf contributes to the greatest

development of the aleurone followed by sodium nitrate, while the thickness of the aleurone is poorest in no-manure plot. Initial soil-fertility differences also contribute to differences in aleurone thickness.

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The writers wish to express their indebtedness to the Imperial Council of Agricultural Research for financial help given in connection with the investigation on 'Quality in Rice'. They are also indebted to Mr. C. R. Srinivasa Ayyangar, Paddy Specialist, for allowing them to make use of the data collected at Coimbatore since the senior author left for Indore.

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TABLE I

Thickness of aleurone layer in white rice varieties of different duration

Strain number	Ripening duration (days)	Thickness of aleurone layer in microns (mean of six readings)					
		Seventh day		Sixteenth day		Thirtieth day	
		Dorsal side	Ventral side	Dorsal side	Ventral side	Dorsal side	Ventral side
Short duration							
Co 10 . .	125	10·0±0·67	7·9±0·7	33·6±1·2	18·0±0·8	29·4±1·1	26·4±1·2
Medium duration							
Co 1	155	9·8±0·6	6·7±0·5	21·0±1·7	13·8±1·7	30·6±2·0	20·4±0·8
Co 7	157	12·0±0·6	..	36·0±3·2	29·0±2·0	36·0±3·3	28·0±0·9
GEB 24 . . .	149	21·0±1·1	8·1±0·4	28·2±1·9	24·6±1·4	26·1±0·6	21·0±0·6
Co 5	164	11·0±0·6	7·7±0·3	25·8±0·6	20·1±0·6	27·0±0·6	13·2±0·7
Long duration							
Co 4	192	..	..	29·4±1·1	16·8±0·6	34·2±1·2	18·0±0·3
Adt 11 . . .	176	..	..	25·8±2·1	15·4±0·7	30·0±1·2	24·9±1·2

TABLE II

Aleurone thickness in white and coloured rices

Variety	Rice colour	Breadthe of the grain in mm.	Coarse or fine	Aleurone thickness in microns (mean of six readings)					
				Seventh day		Sixteenth day		Thirtieth day	
				Dorsal side	Ventral side	Dorsal side	Ventral side	Dorsal side	Ventral side
GEB 24	White	2·1	Fine	21·0±1·1	8·1±0·4	28·2±1·9	24·6±1·4	26·1±0·6	21·0±0·6
Co 4	Do.	2·4	Coarse	..	..	29·4±1·1	16·8±0·6	34·2±1·2	18·0±0·3
Co 1	Do.	2·3	Medium fine	9·8±0·6	6·7±0·5	21·0±1·7	13·8±1·7	30·6±2·0	20·4±0·8
Co 9	Red	2·7	Coarse	8·3±0·5	..	24·5±2·2	15·5±0·8	30·6±1·2	18·0±·01
T 111	Do.	1·8	Fine	7·5±0·3	..	27·3±2·1	11·6±1·2	20·0±1·4	12·0±1·1
T 149	Gold	2·4	Coarse	11·0±0·7	..	20·2±2·4	32·2±2·2	31·6±1·0	29·2±2·1
T 150	Grey-brown	2·9	Do	11·2±0·4	..	23·7±1·3	..	26·0±0·8	19·3±2·2
T 134	Purple	2·7	Do.	8·6±0·4	8·2±0·4	19·8±1·8	22·2±0·7	33·0±3·5	29·2±1·0

TABLE III

Thickness of aleurone layer in glutinous, hard, soft and wild rices

Name of variety	Thickness of aleurone layer in microns (mean of six readings)						Difference significant or not
	Seventh day		Sixteenth day		Thirtieth day		
	Dorsal side	Ventral side	Dorsal side	Ventral side	Dorsal side	Ventral side	
			Glutinous				
1. White puttu (T 304)	16·8±0·8	8·8±0·9	47·4±4·0	21·0±3·0	56·0±3·2	26·0±2·4	Between 1 and 2 significant
2. Black puttu (T 102)	20·2±0·8	15·4±0·5	40·2±1·4	23·4±0·8	48·6±0·8	23·4±0·8	
			Hard				
3. Starch (T 167) .	8·2±0·4	6·8±0·1	36·0±1·9	25·2±2·7	33·3±2·3	25·2±2·1	Between 3 and 4 significant
			Soft				
4. Starch (T 523) .	16·5±0·8	9·5±0·7	28·9±1·2	18·0±0·9	20·7±1·8	19·3±1·4	
			Wild				
5. Starch (T 573) .	9·8±0·7	7·6±0·7	19·0±0·9	9·7±0·6	23·0±2·3	16·0±1·8	Between 3 and 5 significant

TABLE IV

Aleurone thickness of GEB 24 crop raised under different manurial treatments at different places

Place	Manurial treatments	Thickness of aleurone in microns (30 days old) (Mean of 6 readings)	Difference significant or not
Coimbatore	A	17·1±0·6	
	B	19·8±2·0	Over A not significant
	C	17·4±0·3	Ditto.
	D	24·5±1·3	Over A significant
Maruteru (West Godavari) .	A	19·1±1·2	
	B	..	
	C	25·8±2·2	Over A significant
	D	33·1±1·4	Ditto.
Pattambi (Malabar) . . .	A	14·6±1·1	
	B	12·8±0·9	
	C	19·2±1·5	Over A significant
	D	22·2±1·1	Ditto.
Berhampur (Ganjam) . .	A	16·7±0·6	
	B	19·8±0·8	Over A significant
	C	26·4±0·8	Ditto.
	D	25·4±0·1	Ditto.

TABLE V

Aleurone thickness of Co 1 crop in the seed-bed manurial experiments

Treatments	Thickness of aleurone layer in microns (means of six readings)						
	Seventh day		Sixteenth day		Thirtieth day		
	Dorsal side	Ventral side	Dorsal side	Ventral side	Dorsal side	Ventral side	
A . .	9·6±0·7	7·9±0·5	27·0±3·0	14·4±0·01	26·8±1·4	15·3±1·2	Difference highly significant
B . .	9·8±0·6	6·7±0·5	21·3±1·7	13·8±1·7	43·1±1·0	18·0±0·01	

AN INVESTIGATION INTO THE ALGAL-FLORA OF PADDY FIELD SOILS OF THE UNITED PROVINCES, I*

BY

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Research Scholar, Department of Botany, Benares Hindu University

(Received for publication on 14th July 1938)

INTRODUCTION

DURING recent years a good deal of work has been done on the algal-flora of the soil. Among the notable investigations reference may be made to the work of Bristol [1920] on the algae of English soils, of Boye-Petersen [1935] on the algae of the soils of East Greenland and Denmark, and of James [1935] on the algae of natural occurring soils from Redlands woods, Holmwood, Surrey, Shoreham, and Kent. In our country, however, such a work has almost been neglected and the only attempt in this direction has been that of Singh [1933] and Banerji [1935]. The former has worked on the algae of the naturally occurring soils of Lahore, while the latter has investigated the growth of the algae in the soils of an alluvial paddy field of Faridpur, Bengal. Work on the algae of uncultivated soils may not be of any economical value, but investigations of these lower plants from the soils of the Indian crops are likely to be both interesting and important, especially in relation to problems concerning soil biology and agriculture. The writer, therefore, thought it desirable to start investigations on soil algae in this Laboratory. As a beginning the cultivated soils of paddy fields of the United Provinces were taken. The results obtained by the writer are altogether different from those that have been recorded by Banerji, as will be clear from the remarks given at the end of this communication.

TECHNIQUE

What matters greatly in the study of the soil algae is the device for taking up absolutely 'unmixed soil' from various depths. Consequently auger was used in this process and the sampling was done from four different districts of the United Provinces, *i.e.*, Benares, Mirzapur, Gorakhpur and Basti. In all four samples were collected, one from each district. Care has been taken not to sample the soils from isolated paddy fields but from large tracts, covering at least an area of five thousand acres. The samples from Benares were collected on the 18th of October, 1936, near Sakaldiha Station on the

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E. I. Ry. Mirzapur samples were collected on the 9th of September, 1936, near Robertsganj, while those from Gorakhpur were obtained on the 15th of September, 1936, near Ramgarh Tal. The soil samples from Basti were collected on the 30th of September, 1936, near Khalilabad. These samples were taken from five different depths in the following order :—surface, two inches, six inches, twelve inches, and as far as the roots of the paddy plant penetrate the soil, and none was collected from a wet field. The samples were kept in glass specimen tubes and they were left in the vasculum till further use. During the intervals the soils got completely dried up, and this eliminated all such algae as could not resist prolonged desiccation.

Only one series of cultures of each sample were set, the culture medium being an aqueous mineral salt solution with the following composition :—Magnesium sulphate, 0·2 grm., potassium phosphate, 0·2 grm., ammonium nitrate, 0·5 grm., calcium chloride, 0·1 grm. and a trace of ferrous sulphate dissolved in 1,000 c.c. of distilled water. Milk-bottles were used for the cultures and the mouth of each bottle was plugged with cotton wool. Eight empty bottles were heated at a time in an autoclave at a pressure of one and a half atmospheres for fifteen minutes. After twenty-four hours the bottles were subjected to a second heating under the same pressure to ensure complete elimination of bacteria. One hundred and five such bottles were sterilised in this manner and in each one of them 150 c.c. of the culture solution was kept. The bottles were again heated in the autoclave twice in the manner described above. The sterilised solutions were then inoculated on the following day with different amounts of soils (*i.e.*, 10 grms., 5 grms., 2·5 grms., 0·15 grm.) from each of the above-mentioned depths by means of a sterilised spatula. The mouths of all the bottles were then covered with brown paper tied tightly round the neck. The cultures for Mirzapur district were started on the 25th of October, 1936, those for Gorakhpur district on the 30th of October, 1936, those for Basti district on the 5th of November, 1936, and those for Benares district on the 11th of November, 1936. For the convenience of comparison the cultures were named A, B, C and D respectively.

For the study of diatoms the method employed by Bristol [1920] has been followed and this has proved to be very satisfactory. The material was placed in water on a glass-slide and broken up into very small pieces with a pair of needles. The water was allowed to evaporate slowly into the air, the slide being covered with a glass lid to prevent the access of dust. After twenty-four hours' drying the slide was placed in a tube containing boiling strong nitric acid and heated to remove all organic matter. After careful washing in water it was transferred through 95 per cent and absolute alcohol to xylol. The film of the diatoms still remaining attached to the slide was then mounted in Canada balsam. Owing to the minute size of most of these soil-diatoms the markings on the walls were extremely difficult to make out, hence later preparations were made using dammar lac as a mounting medium in order to secure a better definition of the walls.

Growth in culture

The first sign of growth was observed after different intervals, not only in different soils but even in the same soil. At first a white scum which consisted of bacteria gradually appeared on the surface of the culture. This scum next assumed a green tinge, while many green patches began to grow on the surface of the soil below. These patches became large and in due course the whole culture medium assumed a light green colour owing to the formation of free-floating unicells and filaments. The greenish scum also thickened gradually, and the sides of the bottles also became covered with a green or dark green gelatinous membrane. In bottles in which diatoms developed the surface of the soil assumed a dull brown colour.

Different genera and even different species of the same form were observed not to grow simultaneously in culture bottles containing the same type of soil. In the floating scum various unicells, such as, *Chlorococcum*, *Chlorella*, etc., appeared first and these were followed by *Protosiphon* and filaments of *Oscillatoria*. *Phormidium* and *Nostoc* came out next and grew in almost all the bottles. A week later *Lyngbya*, *Phormidium*, *Homoeothrix*, and *Cylindrospermum* made their appearance in succession. At the same time good many of the unicellular and filamentous green algae, *e.g.*, *Spirogyra*, *Oedogonium*, and members of the Chaetophorales grew up. Diatoms appeared early and continued to grow to the last. Moss protonema began to grow very early in nearly all the bottles but disappeared after a very short time. A list of algae found growing in the different bottles containing different samples of soil is given in Tables I-IV.

Growth was luxuriant in those bottles that were left undisturbed for some time, while it was very slow in such as were frequently handled for observations. Throughout November and December, 1936, there was practically no growth in the cultures. There was a slight growth of algae in January, 1937, while in the beginning of February, the growth was plentiful, though in very few species. In the middle of March the cultures showed the climax of growth of the different forms, and it was also noted that the growth was quicker in those bottles that were exposed to direct light than those that were given indirect or diffused light. In April last, however, the bottles had to be placed in diffused light as the direct rays of the sun proved very hot and impeded the growth. In the end of this month the algae began to degenerate and within the first fortnight or so most of the species of the green algae, especially those of *Spirogyra*, disappeared altogether. The *Chlorococcales* and species of *Protosiphon* staggered with the unfavourable conditions for a sufficiently long time, the latter ultimately forming cysts. The blue-green algae were the last to disappear.

As a general observation it may be stated that the algae grew much more favourably in those bottles which contained the least amount of soil, *i.e.*, 0·15 grm.

Systematic enumeration of the species observed growing in the cultures

Myxophyceae

I. Chroococcales

Chroococcaceae

Genus *Chroococcus* Nägeli

1. *Chroococcus minutus* (Kütz.) Näg. Geitler, in Rabenhorst's *Kryptogamenflora von Europa*, Band XIV, Cyanophyceae, 1930-32, p. 234, Fig. 113 *c*.

Lat. colon. cum vag., 8-9·9 μ ; long. colon. cum vag., 16·5-18 μ ; Lat. cell., 3·3-5 μ ; long. cell., 4-6·6 μ ; Lat. cell. cum vag., 4-6 μ ; long. cell. cum vag., 5-9·9 μ.

The alga was found in cultures of all the four soil samples from 11-14 in. depth. It mostly grew at the bottom of the culture solution. Intermingled with this form were filaments of *Oscillatoria* and *Phormidium*.

II. Hormogoneales

Rivulariaceae

Genus *Homoeothrix* (Thuret) Kirchner

2. *Homoeothrix juliana* (Manegh.) Kirchner Geitler, *op. cit.*, 1930-32, p. 576, Fig. 359.

forma *tenuis* form. nov.

Lat. fil., 9·9-12 μ ; lat. trich., 6·6-7 μ ; long. cell., 3·3-4 μ.

The alga was found growing in cultures of samples A, B and D from 8-10 in. depth. It occurred along with *Oscillatoria*, and a large number of forms belonging to *Chlorococcales*.

The present alga differs from the type in possessing narrower trichomes and thicker sheath.

It forms a fairly thick substratum on the surface of the soil.

Genus *Gloeotrichia* Agardh

3. *Gloeotrichia intermedia* (Lemm.) Geitler. var. *kanwaense* Rao Rao, "The Myxophyceae of the United Provinces, India—II", *Proc. Ind. Acad. Sci.*, Vol. III, No. 2, Sec. B, 1936, p. 168, Fig. 2, *A-C*.

Forma

Diam. strat., 1-12 mm. ; lat. trich., at base, 9·9-11 μ higher up, 3-5 μ ; long. cell., at base, 3·3-8 μ ; higher up, 4-9·9 μ ; lat. het., 10-13·5 μ ; long. het., 8-11·5 μ ; lat. spor. cum vag., 13-15 μ ; lat. spor., 12-15 μ ; long. spor., 54·5-63 μ ; crass. vag., 0·8-1 μ.

This alga appeared in many of the cultures of all the four samples from 2-8 in. depth together with *Nostoc* and *Protosiphon*. The growth consisted of pale yellowish green rounded masses on the surface of the soil.

The thicker sheath, at the base of the filament above the spore, in which the alga differs from the type is possibly due to the disintegration of the vegetative cells there.

Nostocaceae

Genus *Cylindrospermum* Kuetzing

4. *Cylindrospermum muscicola* Kütz. Frémy. "Les Myxophycées de Madagascar," *Annales de Cryptogamie Exotique*, t. iii. fasc. IV, 1930, p. 377, Fig. 313; Tilden, *Minnesota Algae*, Vol. I, 1910, pl. X, Fig. 6; Ghose, "On some Myxophyceae from Rangoon", *Jour. Burma Res. Soc.*, Vol. XV, Part III, 1926, pl. VII, Fig. 15.

Forma

Lat. cell., 3·3-4·6 μ; long. cell., 4·5-6·6 μ; lat. het., 3·3-5 μ; long. het. 6·6-7·8 μ; lat. spor., 9·2-12·6 μ; long. spor., 13·2-20·3 μ.

The alga appeared in many of the cultures of all the four samples from 2-8 in. depth. It forms a dark brown patch on the surface of the soil.

It differs from the type in possessing slightly narrower and longer heterocysts and in the longer cells.

Genus *Nostoc* Vaucher

5. *Nostoc carneum* forma minor Bhâradwâja Bhâradwâja, "The Myxophyceae of the United Provinces, India.—I," *Proc. Ind. Acad. Sci.*, Vol. II, No. 1, Sect. B, 1935, p. 102.

Lat. trich., 3-4 μ; lat. het., 4·6-5·4 μ; long. het., 4·2-6·4 μ; lat. spor. 4-6·4 μ; long. spor., 5-9·9 μ.

The alga appeared in many of the cultures of all the samples from 2-6 in. depth. It forms a pale green mass on the surface of the soil.

6. *Nostoc ellipsosporum* Rabenh. Geitler, *op. cit.*, 1930-32, p. 842, Fig. 533.

Lat. cell., 3-3·3 μ; long. cell., 3·3-8 μ; lat. het., 4·4-6·6 μ; long. het., 8·2-9·9 μ; lat. spor., 6·6-8·5 μ; long spor., 10-13·2 μ.

The alga appeared in nearly most of the cultures of all the samples from 8 in. depth. It forms spherical to formless, strong, spreading, olive-green to green plant mass on the surface of the soil.

Oscillatoriaceae

Genus *Oscillatoria* Vauch.

7. *Oscillatoria terebriformis* Ag. Geitler, *op. cit.*, 1930-32, p. 955, Fig. 607, *d*.

Lat. trich., 4·5-6·6 μ; long. cell., 1·7-4·6 μ.

The alga was found growing together with sterile filaments of *Oedogonium* in many of the cultures in all the samples from 2 in. depth. It forms a thin blue green covering over the surface of the soil. It differs from the type in possessing shorter cells.

8. *Oscillatoria variabilis* Rao Rao *op. cit.*, 1936, p. 172, Fig. 3, *A-D.*

Forma

Lat. trich., 6·2-6·6 μ ; long. cell., 3-3·3 μ.

The form possesses slightly broader trichomes. It differs from the type in having only rounded apical cells.

The alga was found in cultures of all the four samples from 11-14 in. depth. It mostly grew at the bottom of the culture solution intermingled with filaments of *Phormidium luridum* (Kütz) Gom., and other species of *Oscillatoria.*

9. *Oscillatoria proboscida* Gom. Geitler, *op. cit.*, 1930-32, p. 943, Fig. 598, *b.*

Lat. trich., 13·2-14 μ ; long. cell., 3·3-4 μ.

The alga appeared in several of the cultures of all the samples from 11-14 in. depth together with *Phormidium luridum* (Kütz.) Gom. and *Oscillatoria variabilis* Rao.

10. *Oscillatoria quadripunctulata* var. *unigranulata* Singh Singh, "The Myxophyceae of the United Provinces, India. IV".—*Proc. Ind. Acad. Sci.*, 1939, Vol. 9, No. 1, Fig. I, *F.*

Lat. trich., 1-1·7 μ ; long. cell., 7-8·3 μ.

The alga appeared in many of the cultures of all the samples from 11-14 in. depth together with other species of *Oscillatoria* and *Phormidium.*

The alga differs from the type in the possession of longer cells and single granule on either side of the septum.

Genus *Phormidium* Kütz.

11. *Phormidium Retzii* (Ag.) Gom. Geitler, *op. cit.*, 1930-32, p. 1012, Fig. 647, *a-d.*

Lat. fil., 12-13·2 μ ; lat. trich., 8-9·9 μ ; long. cell., 4·9-9·9 μ.

The alga was found growing in all the samples and in several of the cultures from 2-6 in. depth. It forms a dark bluish green thin layer over the surface of the soil.

The alga differs from the type in possessing indistinct tapering end-cells without any membrane.

12. *Phormidium luridum* (Kütz.) Gom. Geitler, *op. cit.*, 1930-32, p. 1010, Fig. 645, *a.*

Lat. trich., 1·5-3 μ ; long. cell., 1·5-4·2 μ.

The alga was found growing in many of the cultures of all the samples from 11-14 in. depth together with *Chroococcus minutus* (Kütz.) Näg. and filaments of *Oscillatoria*. It forms a blue green layer on the surface of the soil.

The alga differs from the type in the dimensions and in possessing straight tapering end-cells.

Genus *Lyngbya* C. Agardh

13. *Lyngbya limnetica* Lemm. Geitler, *op. cit.*, 1930-32, p. 1045, Fig. 661, *a-b*.

Lat. fil., 2-3·3 μ ; lat. trich., 1·5-1·7 μ ; long. cell., 1·99-2·49 μ.

The alga was found growing in many of the cultures of A, C and D samples from 12-14 in. depth. If forms a purple thin layer on the surface of the soil.

The alga differs from the type in having longer cells and in the end-cells being slightly tapering.

Genus *Microcoleus* Desmazierea

14. *Microcoleus chthonoplastes* Thuret. Geitler, *op. cit.*, 1930-32, p. 1134, Fig. 739 ; Bhâradwâja, " The Myxophyceae of the United Provinces, India.—I, " *Proc. Ind. Acad. Sci.*, Vol. II, No. 1, Sec. B, 1935, p. 98, Fig. 2, *b* and *c*.

Lat. cell., 3·4-4 μ ; lat. vag., up to 20 μ.

This alga appeared together with filaments of *Oscillatoria* in nearly most of the cultures from 6 in. depth. It forms a beautiful bluish green stratum on the surface of the soil.

Chlorophyceae

Volvocales

Chlamydomonadineae

Chlamydomonadaceae

Genus *Chlamydomonas* Ehrenberg

1. *Chlamydomonas intermedia* Chodat Pascher, in Pascher's *Süsswasserflora Deutschlands, Österreichs und Der Schweiz.*, Heft 4, 1927, p. 202, Fig. 141, *a-r*.

Lat. cell., 12-16 μ ; long. cell., 18-25 μ.

The alga appeared in many of the cultures of all the samples from 2-6 in. depth.

Chlorococcales

Chlorococcaceae

Genus *Chlorochytrium* Cohn.

2. *Chlorochytrium paradoxum* (Klebs) G. S. West James, " An investiga-

tion of the algal growth in some naturally occurring soils" *Beiheften zum Botanischen Centralblatt*, Bd. LIII, Abt. A, Heft 3, 1935, p. 527, Fig. 3, *A-E*.

Diam. smaller cells., 9·9-13·2 μ ; diam. larger cells., 23·3-30 μ ; lat. spor., 3·3-4·9 μ ; long. spor., 6·6-8 μ.

The alga was found growing in nearly all the cultures of all the samples from 2-6 in. depth in association with *Chlorella vulgaris* Beyerinck and *Chlorococcum infusionum* (Schrank) Meneghini. It forms a green papery substratum upon the surface of the culture solution.

Genus *Characium* A. Braun

3. *Characium nasutum* Rabenhorst Lemmermann, Brunnthaler und Pascher in Pascher's *Süsswasserflora Deutschlands, Österreichs und Der Schweiz*, 1915, Heft 5, p. 79, Fig. 15.

Lat. cell., 18-20 μ ; long. cell., 40-70 μ.

The alga was found growing as an epiphyte on *Oedogonium* filaments in many of the cultures of all the samples from 2 in. depth. Sometimes it forms a cluster. It differs from the type in possessing smaller cells.

4. *Characium acuminatum* A. Braun Lemmermann, Brunnthaler und Pascher, *op. cit.*, 1915, Heft 5, p. 79, Fig. 18.

Lat. cell., 10-15 μ ; long. cell., 20-35 μ.

The alga was found growing in many of the cultures of all the samples from 2-6 in. depth in association with *Chlorococcum infusionum* (Schrank) Meneghini. and *Chlorella vulgaris* Beyerinck.

Genus *Chlorococcum* Fries.

5. *Chlorococcum infusionum* (Schrank) Meneghini. Lemmermann, Brunnthaler und Pascher, *op. cit.*, 1915, Heft. 5, p. 63, Fig. 2.

Diam. cell., 15-40 μ ; lat. spor., 2-3 μ ; long. spor., 5-6 μ.

The alga appeared in nearly all the cultures of all the samples from 2-6 in. depth. It forms a fairly thick green layer upon the surface of the culture solution. It is found in association with *Chlorochytrium paradoxum* (Klebs) G. S. West and *Chlorella vulgaris* Beyerinck.

Chlorellaceae

Genus *Chlorella* Beyerinck

6. *Chlorella vulgaris* Beyerinck Lemmermann, Brunnthaler und Pascher, *op. cit.*, 1915, Heft 5, p. 112, Fig. 71.

Diam. cell., 6·6-9 μ (12 μ).

The alga was found growing in nearly all the cultures of all the samples from 2-6 in. depth. It forms a green layer upon the surface of the culture solution together with *Chlorochytrium paradoxum* (Klebs) G. S. West and *Chlorococcum infusionum* (Schrank) Meneghini.

Genus *Trochiscia* Kütz.

7. *Trochiscia reticularis* (Reinsch.) Hansg. James, *op. cit.*, 1935, p. 527, Fig. 3, *H-K*.

Diam. cell., 12-18 (up to 28) μ.

The alga appeared only in cultures of sample A from 2 in. depth. The cells were found scattered as well as aggregated to form a palmelloid colony in association with *Chlorococcum* and *Chlorella*.

The alga agrees with the type described by James in the diameter of the cells, in possessing single irregular reticulate chloroplast with a single pyrenoid. But, it differs from the same in the following points :—

(i) In all the cells (young and old cells) there is a third smooth laver external to the sculptured-wall.

(ii) The sculptured-wall consists of a network of regular ridges.

The palmelloid state and the autospores have been noticed.

Hydrodictyaceae

Genus *Pediastrum* Meyen.

8. *Pediastrum clathratum* var. *duodenarium* (Bailey) Lemmermann Lemmermann, Brunnthaler und Pascher, *op. cit.*, 1915, Heft 5, Fig. 56, *C*.

Colon. of 12-18 cells., diam. colon., 45-50 μ.

The alga was found growing among the *Oedogonium* filaments in many of the cultures of all the samples from 2 in. depth.

Chaetophorales

Trentepohliaceae

Gongrosireae

Genus *Gongrosira* Kütz.

9. *Gongrosira terricola* Bristol Bristol, "On the Alga-Flora of some Desiccated English Soils," *Ann. Bot.*, No. CXXXIV, 1920, Pl. II.

Lat. cell., 7-15 μ ; long. cell., 10-18 μ ; diam. zoogonidang., 15-18 μ.

The alga appeared in many of the cultures of all the samples from 12-14 in. depth. It forms a thin papery incrustation on the surface of the culture solution together with *Protosiphon botryoides* (Kützing) Klebs forma *parieticola* Iyengar.

It agrees with the type in forming an expanded stratum, the creeping filaments of which are closely interwoven to form a pseudo-parenchymatous disc of distended cells, in short and tapering upright filaments with obtuse apices and irregular branching, in possessing a band-shaped chloroplast with one to two pyrenoids and in sub-spherical basal or rarely intercalary zoogonidangia.

10. *Gongrosira circinnata* (Borzi) Schmidle Heering in Pascher's *Süsswasserflora Deutschlands, Österreichs und Der Schweiz*, 1914, Heft 6, p. 112, Fig. 161, *a & b*.

Diam. cell., 9·9-12 μ.

The alga appeared together with *Gongrosira terricola* Bristol and *Protosiphon botryoides* (Kützing) Klebs forma *parieticola* Iyengar in many of the cultures of all the samples of soil from 12-14 in. depth. The branching filaments of this alga appeared to come out from the pseudo-parenchymatous discs of *Gongrosira terricola* Bristol.

Genus *Pleurastrum* Chodat

11. *Pleurastrum constipatum* Printz Printz, "Subäerial Algae from South Africa," *Norske Videnskabers Selskabs Skrifter*, 1920, Nr. I, Pl. III, Fig. 124-155.

Diam. cell., 7-8·9 μ.

The alga was found growing together with *Gongrosira terricola* Bristol and *Protosiphon botryoides* forma *parieticola* Iyengar in many of the cultures of all the samples from 12 in. depth. It forms a green to yellowish green mucilaginous stratum on the surface of the culture solution.

12. *Pleurastrum insigne* Chod. Heering, *op. cit.*, 1914, Heft 6, p. 112, Fig. 166, *a*, *b* & *c*.

Lat. cell., 5-8 μ ; long. cell., 7-10 μ.

The alga was found growing together with *Chlorella vulgaris* Beyerinck and *Chlorococcum infusionum* (Schrank) Meneghini in many of the cultures of all the samples from 2 in. depth. It forms a mucilaginous green papery layer on the surface of the culture solution.

Oedogoniales

Genus *Oedogonium* Link

13. *Oedogonium intermedium* Wittrock Hirn, *Monographie und Iconographie der Oedogoniaceen*, 1900, p. 94, Pl. V, Fig. 31 ; Tiffany, *The Oedogoniaceae*, Ohio, 1930, p. 72, Pl. XIV., Fig. 134.

Forma

Lat. cell., 14·2-16·5 μ ; long. cell., 30-37 μ ; crass. oog., 26·4-30 μ ; long. oog., 30-34 μ ; crass. oosp., 26·4-27 μ ; long. oosp., 26·4-27 μ ; lat. anth., 13·2-14·5 μ ; long. anth., 6·6-13 μ.

The alga was found growing with filaments of *Spirogyra* in many of the cultures of all the samples from 2-6 in. depth. It differs from the type in smaller dimensions.

14. *Oedogonium hirnii* Gutwinski Hirn, *op. cit.*, 1900, p. 93, Pl. V, Fig. 29 ; Tiffany, *op. cit.*, 1930, p. 73, Pl. XIV, Figs. 136 and 137.

Forma

Lat. cell., 9·9-13·2 μ ; long. cell., 33·3-56 μ ; crass. oog., 33-34 μ ; long. oog., 33·3-40 μ ; crass. oosp., 26·4-28 μ ; long. oosp ; 26·4-28 μ ; crass. anth., 9·9-10·5 μ ; long. anth., 3·3-4·2 μ.

The alga was found growing in many of the cultures from 2-6 in. depth. The form differs from the type in smaller oospores and antheridia.

Conjugales

Euconjugateae

Zygnemaceae

Genus *Spirogyra* Link

15. *Spirogyra* sp.

Lat. cell., 45-50 μ ; long. cell., 225-230 μ.

The alga was found growing together with *Oedogonium* and other spp. of *Spirogyra* in nearly all the cultures of all the samples of soil from 2 in. depth.

The vegetative cells are 5-6 times as long as broad ; end-walls are replicate and chloroplasts are four in number.

The zygospores were not formed, hence the sp. could not be determined.

16. *Spirogyra* sp.

Lat. cell. 22·5-25 μ ; long. cell., 210-225 μ.

The alga was found growing together with other spp. of *Spirogyra* in many of the cultures of all the samples from 2 in. depth.

The vegetative cells are 5-6 times as long as broad ; end-walls are replicate and the chloroplast is single.

The zygospores were not formed, hence the species could not be determined.

Siphonales

Protosiphonaceae

Genus *Protosiphon* Klebs

17. *Protosiphon botryoides* (Kütz.) Klebs forma *parieticola* Iyengar Iyengar, "On an Indian form of *Protosiphon botryoides* Klebs, "*Archiv fur Protistenkunde*, Band 79, Heft 2, 1933, Fig. 1, *A-Z*.

Lat. vesicl., 40-60 μ ; long. vesicl., 80-100 μ ; lat. rhizoid., 10-36 μ ; long. rhizoid., 90-150 μ ; diam. cyst., 20-30 μ.

The alga was found growing in all the cultures of all the samples from 12-14 in. depth. It forms a papery gelatinous green scum on the surface of the culture solution.

The alga differs from the type in possessing shorter and somewhat broader rhizoidal portion and in the absolute absence of lamellation in the walls.

Bacillariophyceae

Pennales

Araphidineae

Fragilariaceae

Tabellarioideae

Fragilarioideae

Genus *Synedra* Ehrenberg

1. *Synedra ulna* var. *amphirhynchus* (Ehr.) Grun. Hustedt, "Die Kieselagen, Lieferung 1, and 2 in L. Rabenhorst's *Kryptogamen-Flora Deutschlands, Osterreich u. d. Schweiz*, B. VII, Leipzig, 1931-32, p. 197, Fig. 691 A, *e*.

Lat. frust., 6·6-9·9 μ ; long. frust., 118-200 μ ; striae, 10-12 in 10 μ.

2. *Synedra ulna* var. *spathulata* Grun. Hustedt, *op. cit.*, 1932, p. 197, Fig. 691A, *h*.

Lat. frust., 13·2-16·5 μ ; long. frust., 230-244 μ ; striae, 9-10 in 10 μ.

Biraphidineae

Naviculaceae

Naviculoideae

Genus *Anomoeoneis* Pfitzer

3. *Anomoeoneis sphaerophora* (Kütz.) Printz. West and Fritsch, "The British Freshwater Algae," Cambridge, 1927, p. 372, Fig. 156, *c*.

Lat. frust., 8-9·9 μ ; long. frust., 30-35 μ.

Genus *Navicula* Bory

4. *Navicula radiosa* var. *acuta* Grun. Majeed, "The Freshwater Algae of the Punjab," Part I, 1935, Pl. II, Fig. 19.

Lat. frust., 6·6-7 μ ; long frust., 35-36·3 μ ; striae, 10-12 in 10 μ.

Genus *Pinnularia* Ehrenberg

5. *Pinnularia interrupta* Smith Smith, "A Synopsis of the British Diatomaceae," Vols. I and II, London, 1853, p. 59, Pl. XIX, Fig. 184.

Lat. frust., 4·9-5·3 μ ; long. frust., 29·7-30·5 μ ; striae, 10 in 10 μ.

The alga differs from the type in possessing smaller dimensions.

6. *Pinnularia major* Smith Smith, *op. cit.*, 1853, p. 54, Pl. XVIII, Fig. 162.

Forma

Lat. frust., 9·9-10·5 μ ; long. frust., 59-65 μ ; striae, 12-13 in 10 μ.

The form differs from the type in smaller dimensions.

7. *Pinnularia subcapitata* Greg. var. *paucistriata*. James, *op. cit.*, Heft 3, 1935, p. 545, Fig. 12, *C-D*.

Lat. frust., 4·5-5 μ ; long. frust., 32-34 μ ; striae, 9-10 in 10 μ.

Gomphocymbelloideae

Genus *Amphora* Ehrenberg

8. *Amphora ovalis* var. *gracilis* V. H. Majeed, *op. cit.*, 1935, Pl. IV, Fig. 7.

Lat. frust., 10-12 μ ; long. frust., 16·5-20 μ ; striae. 10-14 in 10 μ.

Genus *Cymbella* Agardh

9. *Cymbella Ehrenbergii* Kütz. Smith, *op. cit.*, 1853, p. 17, Pl. II, Fig. 21.

Lat. frust., 19·5-20 μ ; long. frust., 85-90 μ ; striae, 6-7 in 10 μ.

10. *Cymbella ventricosa* Kütz. Majeed, *op. cit.*, 1935, Pl. III, Figs. 11, 12 and 13.

Lat. frust., 6·5-7 μ ; long. frust., 23-25 μ ; striae, 10-12 in 10 μ.

Epithemiaceae

Rhopalodioideae

Genus *Rhopalodia* O. Muller

11. *Rhopalodia gibba* (Ehr.) O. Mull. West and Fritsch, *op. cit.*, 1927, p. 381, Fig. 163, *A*.

TABLE I

Date of inoculation, October 26, 1936; Date of examination, January 20, 1937; Depth to which the roots of paddy plants reach the soil, 13-15 in.

Names of algae growing	Surface				2 in.				6 in.				12 in.				As far as the roots of paddy plants reach the soil			
	10 grm.	5 grm.	2·5 grm.	0·15 grm.	10 grm.	5 grm.	2·5 grm.	0·15 grm.	10 grm.	5 grm.	2·5 grm.	0·15 grm.	10 grm.	5 grm.	2·5 grm.	0·15 grm.	10 grm.	5 grm.	2·5 grm.	0·15 grm.
Chroococcus minutus															×	×		×		×
Homoeothrix juliana														×		×	×		×	×
Gloeotrichia intermedia						×		×		×	×	×								
Cylindrospermum muscicola			×		×		×	×			×	×								
Nostoc carneum											×	×			×	×	×	×	×	×
Nostoc ellipsosporum											×	×			×	×	×	×	×	×
Oscillatoria terebriformis		×			×		×	×												
Oscillatoria variabilis													×		×	×		×	×	×
Oscillatoria proboscida													×	×	×		×		×	×
Oscillatoria quadripunctulata var. *unigranulata*														×	×	×		×	×	×
Phormidium Retzii													×	×	×	×		×	×	×
Phormidium luridum														×	×		×		×	×
Lyngbya limnetica													×		×	×		×	×	×
Microcoleus chthonoplastes														×	×	×		×	×	×
Chlamydomonas intermedia				×		×	×	×	×		×	×								
Chlorochytrium paradoxum					×	×		×		×	×	×								
Characium nasutum						×	×	×												
Characium acuminatum							×	×		×	×	×								
Chlorococcum infusionum						×	×	×		×	×	×								
Chlorella vulgaris							×	×	×	×	×	×								
Trochiscia reticularis					×	×	×	×												
Pediastrum clathratum var. *duodenarium*					×	×	×	×												

| | | |

TABLE II (Concluded)

Date of inoculation, October 30, 1936 ; Date of examination, January 23, 1937 ; Depth to which the roots of paddy plants reach the soil, 14-18 in.

Names of algae growing	Surface				2 in.				6 in.				12 in.				As far as the roots of the paddy plants reach the soil			
	10 grm.	5 grm.	2·5 grm.	0·15 grm.	10 grm.	5 grm.	2·5 grm.	0·15 grm.	10 grm.	5 grm.	2·5 grm.	0·15 grm.	10 grm.	5 grm.	2·5 grm.	0·15 grm.	10 grm.	5 grm.	2·5 grm.	0·15 grm.
Chroococcus minutus														×	×	×		×	×	×
Homoeothrix juliana														×	×	×				
Gloeotrichia intermedia						×	×	×		×		×				×	×			
Cylindrospermum muscicola							×	×			×	×	×		×	×				
Nostoc carneum										×	×	×		×	×	×				
Nostoc ellipsosporum										×	×	×	×	×	×	×				
Oscillatoria terebriformis			×	×		×	×	×			×	×			×	×				
Oscillatoria variabilis												×		×	×	×		×	×	×
Oscillatoria proboscida								×		×		×	×	×	×	×		×	×	×
Oscillatoria quadripunctulata var. *unigranulata*						×	×		×		×	×		×	×	×		×	×	×
Phormidium Retzii						×	×	×		×	×	×		×						
Phormidium luridum														×	×	×	×		×	×
Lyngbya limnetica							×	×		×	×	×								
Microcoleus chthonoplastes							×	×	×		×	×	×	×						
Chlamydomonas intermedia					×		×	×	×	×	×	×								
Chlorochytrium paradoxum						×	×	×	×	×	×	×								
Characium nasutum						×	×	×			×	×								
Characium acuminatum					×		×	×		×	×	×								
Chlorococcum infusionum						×	×	×	×	×	×	×								
Chlorella vulgaris						×	×	×			×	×								
Trochiscia reticularis																				
Pediastrum clathratum var. *duodenarium*		×		×		×	×	×												

| | | |

TABLE III (Contd.)

Date of inoculation, November 5, 1936 ; Date of examination, January 28, 1937 ; Depth to which the roots of paddy plants reach the soil, 13-17 in.

Names of algae growing	Surface				2 in.				6 in.				12 in.				As far as the roots of the paddy plants reach the soil			
	10 grm.	5 grm.	2·5 grm.	0·15 grm.	10 grm.	5 grm.	2·5 grm.	0·15 grm.	10 grm.	5 grm.	2·5 grm.	0·15 grm.	10 grm.	5 grm.	2·5 grm.	0·15 grm.	10 grm.	5 grm.	2·5 grm.	0·15 grm.
Chroococcus minutus											×	×		×	×	×		×	×	×
Homoeothrix juliana																				
Gloeotrichia intermedia						×	×	×	×		×	×			×	×				
Cylindrospermum muscicola							×	×			×	×		×						
Nostoc carneum					×		×	×	×	×	×	×								
Nostoc ellipsosporum										×	×	×		×	×	×				
Oscillatoria terebriformis									×		×	×								
Oscillatoria variabilis		×		×		×	×	×												
Oscillatoria proboscida														×	×	×	×	×	×	×
Oscillatoria quadripunctulata var. *unigranulata*			×	×	×					×	×		×		×	×		×	×	×
Phormidium Retzii		×	×			×	×	×	×	×	×	×								
Phormidium luridum														×	×	×		×	×	×
Lyngbya limnetica														×	×	×	×	×	×	×
Microcoleus chthonoplastes															×	×		×	×	×
Chlamydomonas intermedia						×	×	×	×	×	×	×				×		×		
Chlorochytrium paradoxum							×	×		×		×		×	×					
Characium nasutum		×		×	×	×	×	×												
Characium acuminatum							×	×		×	×	×								
Chlorococcum infusionum						×	×	×	×		×	×	×							
Chlorella vulgaris					×	×	×	×	×		×	×		×	×					
Trochiscia reticularis																				
Pediastrum clathratum var. *duodenarium*			×	×		×	×	×												

| | | |

TABLE IV

Date of inoculation, November 11, 1936; Date of examination, January 30, 1937; Depth to which the roots of paddy plants reach the soil, 14-20 in.

Names of algae growing	Surface				2 in.				6 in.				12 in.				As far as the roots of the paddy plants reach the soil			
	10 grm.	5 grm.	2·5 grm.	0·15 grm.	10 grm.	5 grm.	2·5 grm.	0·15 grm.	10 grm.	5 grm.	2·5 grm.	0·15 grm.	10 grm.	5 grm.	2·5 grm.	0·15 grm.	10 grm.	5 grm.	2·5 grm.	0·15 grm.
Chroococcus minutus										×		×			×	×		×	×	×
Homoeothrix juliana							×	×			×	×		×	×	×				
Gloeotrichia intermedia						×	×	×			×	×	×	×						
Cylindrospermum muscicola						×	×	×		×	×	×								
Nostoc carneum										×	×	×								
Nostoc ellipsosporum										×	×	×			×	×				
Oscillatoria terebriformis	×	×		×			×	×		×	×									
Oscillatoria variabilis												×			×				×	×
Oscillatoria proboscida											×			×	×			×	×	×
Oscillatoria quadripunctulata var. *unigranulata*														×	×	×		×	×	×
Phormidium Retzii											×	×						×	×	×
Phormidium luridum																		×	×	×
Lyngbya limnetica																		×	×	×
Microcoleus chthonoplastes										×	×	×								
Chlamydomonas intermedia			×	×						×	×	×								
Chlorochytrium paradoxum									×			×								
Characium nasutum		×		×			×	×												
Characium acuminatum										×	×	×								
Chlorococcum infusionum									×		×	×								
Chlorella vulgaris							×	×			×	×								
Trochiscia reticularis																				
Pediastrum clathratum var. *duodenarium*				×		×	×	×												

| | | |

Nitzschiaceae

Nitzschioideae

Genus *Nitzschia* Hassall

12. *Nitzschia palea* (Kütz.) W. Smith Majeed, *op. cit.*, 1935, Pl. VI, Figs. 2 & 3.

Lat. frust., 3·3-4 μ ; long. frust., 29·7-30 μ.

CONCLUDING REMARKS

In comparing the algal growth in these cultures with that obtained by Banerji [1935], a marked degree of dissimilarity is apparent.

In the first place, Banerji has not made any mention of the depth from which he sampled his soils. It seems from his statements that the soils were taken from the surface and in one case he has actually made cultures of mud. The value of the study of these algae with regard to their economy in soil and agriculture lies in the depth of the soil to which they grow—a point which has altogether been ignored by Banerji. The investigations of the writer [1939] on the Myxophyceae of U. P. and also those of Rao [1937] have conclusively shown that spp. of *Calothrix*, *Nostoc*, *Phormidium* and *Oscillatoria* are the commonest amongst the blue-greens found on the surface of the soil and in muddy waters. It is, therefore, natural that Banerji found *Calothrix Braunii*, *Nostoc* sp., *Phormidium corium* and P. *valderianum* in his soil cultures.

Secondly, Banerji has completely overlooked the conditions of the soil from which he set his cultures. He has taken into consideration the wet soils. The idea of such investigations is to bring out those algae which can resist prolonged desiccation and so can live normally under the surface of the soil for long periods. This fact is constantly emphasized by other workers on soil algae, especially by Bristol [1920], who left her soil samples for four to twenty-six weeks for drying before actually setting the cultures.

Thirdly, a symbiotic relationship between the algae and bacteria of the soil, a fact, which had long been stated by Kossowitsch [1894] and to which Banerji has referred to, is very much doubted at the present time. Fritsch [1936] is of opinion that " the algae in relation to nitrogen fixation beneath the illuminated zone of the soil must depend, in large measure, on their being in a condition of active growth and multiplication, and of that he believes no adequate proof has so far been presented ". He has also remarked that " a study of the surface community, therefore, should include not only the forms visible at the surface, but also those encountered at depths to which light is found to penetrate. It is not improbable that a microstratification of the

surface-living soil algae may be found to obtain, depending upon their immediate light requirements and upon their relative degree of susceptibility to desiccation. No data are at present available concerning the depth to which light can penetrate beneath the surface of a compact soil, but it is doubtful whether there is anything but darkness below the top few centimeters ".

Fourthly, Banerji reports that his cultures are especially rich in blue-green algae. He mentions the complete absence of diatoms and records only one member of the green algae, *i.e., Pleurococcus Naegelii.** The writer, during his investigations, has observed good many of the Myxophyceae growing, though represented by different forms from those mentioned by Banerji, excepting one, *i.e., Phormidium luridum.* Again, the universal and widespread occurrence of diatoms has been noticed, as also of the large number of the Chlorophyceae.

Summary

The investigation by means of water cultures of four samples of soil from paddy fields of widely separated localities of the United Provinces has shown that there is a widely distributed ecological plant formation of moss protonema and algae.

The most important algae in this formation are *Chlorella vulgaris, Trochiscia reticularis, Gongrosira terricola, Oedogonium intermedium* and *Protosiphon botryoides* forma *parieticola.*

The total number of species and varieties found in these soils is forty-three, out of which are fourteen Myxophyceae, seventeen Chlorophyceae and twelve Bacillariophyceae. The soil samples had all been subjected to desiccation for four to five weeks before they were placed in the cultures.

In conclusion, I have much pleasure in expressing my great indebtedness to Professor Y. Bhâradwâja for his kind guidance and criticism throughout the course of this investigation.

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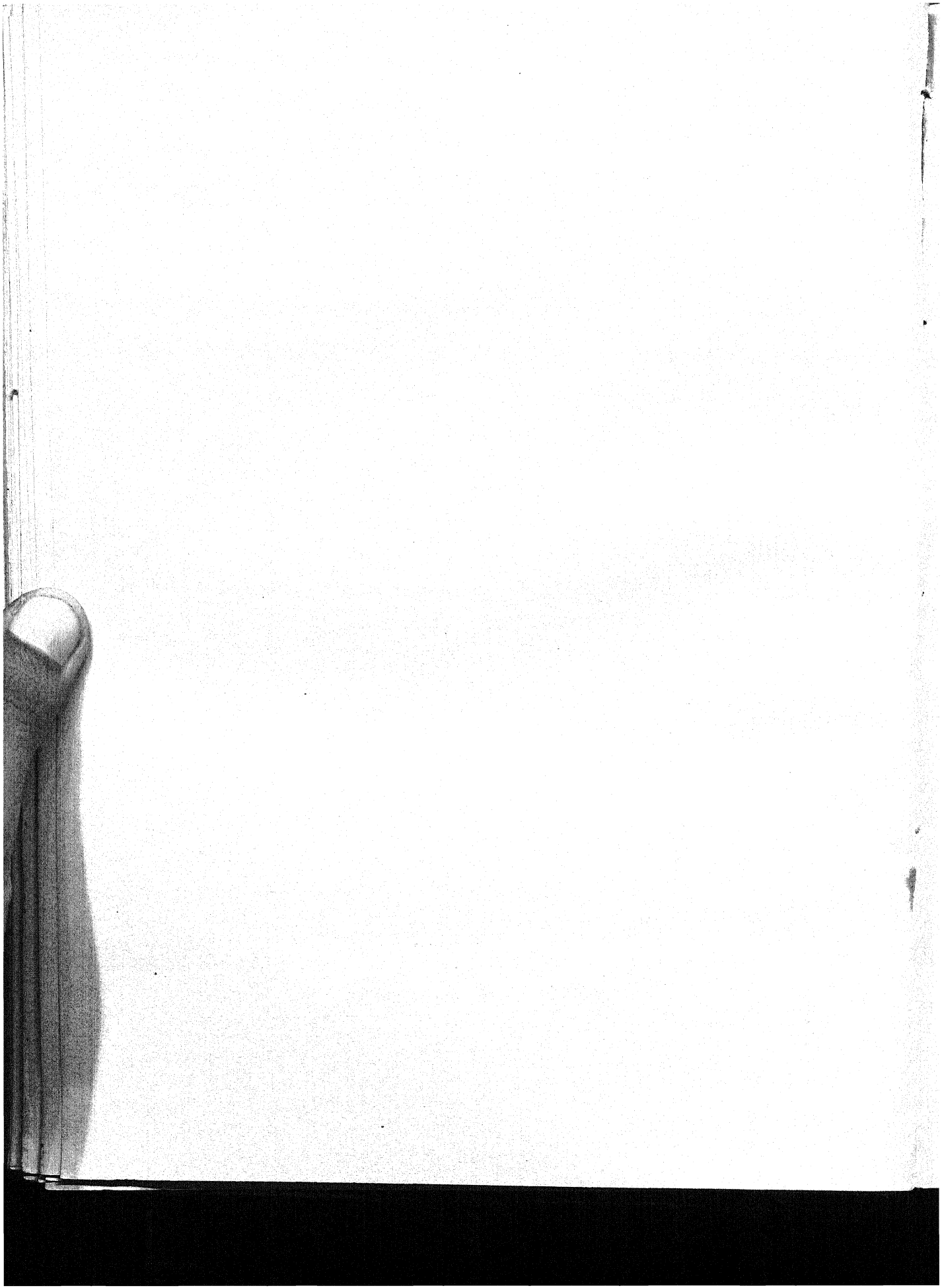
* Banerji has written the name *Protococcus viridis*, but this is not in vogue at present. The current name is *Pleurococcus Naegelii* (*cf.* Fritsch " The Structure and Reproduction of Algae, " 1935, p. 289).



ON THE PROBABILITY OF SEASONAL MIGRATION AMONG THE SOLITARY PHASE INDIVIDUALS OF *LOCUSTA MIGRATORIA* IN NORTH-WEST INDIA

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(With Plate V and one map)

Introduction

In connection with a scheme of Locust Research, which has been in progress since 1931 under the auspices of the Imperial Council of Agricultural Research in India, intensive surveys of various desert areas of Sind, Baluchistan, Rajputana and South-West Punjab are being carried out periodically by a special staff, with the object of studying the seasonal distribution and activities of the solitary phase of the Desert Locust (*Schistocerca gregaria* Forsk.) in North-West India. In the course of these surveys, the survey staff have in addition to the Desert Locust been meeting with solitary phase individuals of two other locusts, *viz.*, *Locusta migratoria* ph. *solitaria* (Migratory Locust), and *Patanga succincta* (Bombay Locust). No particular significance was, however, attached to this circumstance during the earlier years of the work, as it was generally thought that their presence, as indeed in the case of individuals of even the Desert Locust, was merely of an incidental character, presumably indicating the occurrence of local breeding and multiplication as a result of the development of favourable ecological conditions.

During the year 1935, however, fairly conclusive evidence was obtained as to the capacity of the solitary phase individuals of the Desert Locust to migrate long distances at different seasons of the year, whereby they generally succeeded in reaching suitable breeding places in a different rain-belt [Rao, 1936 and 1937]. Thus, *solitaria* individuals that had bred in spring in Baluchistan, or perhaps even in Iran, were, during that year, noticed appearing suddenly in the Sind-Rajputana areas in June-July, while the generation produced in the Indian Desert areas during summer and autumn of that year was observed gradually leaving these regions and migrating into Mekran during autumn and winter months. In the Sind-Rajputana regions, which is subject to summer rainfall, the Desert Locust population was found to be at its minimum during the dry months of the year, *viz.*, January to May; while, in the regions of winter rainfall, especially in the hinterland of Baluchistan, the

minimum was reached during July to September. Detailed observations, continued during the years following, *viz.*, 1936 and 1937, have served to confirm the deductions made as to the existence of normal seasonal migration from one rain-belt to another in the Indo-Iranian areas, among the solitary phase individuals of *Schistocerca gregaria*.

The analogy of the deductions referred to above suggested the idea that the occasional records of captures of stray specimens of the Migratory Locust and the Bombay Locust might perhaps be attributable to the occurrence of a somewhat similar migration of the *solitaria* individuals from one part of the country to another. All the data recorded during the past few years, in regard to the occurrence of these two species of locusts in their *solitaria* phase in the regions under regular observation, were, therefore, collected together, analysed and correlated with meteorological and other data. It is the aim of this note to set forth the inferences made as a result of these studies.

Available data for the Bombay Locust, *Patanga succincta* L

All the information available has been classified in Table I (Appendix), from a study of which it would be seen that the data on hand are comparatively scanty. It is, however, rather significant that all the specimens had been collected either from the Sind-Rajputana Desert areas or from the Cutch-Kathiawar region, and that none had been found in the Baluchistan region with the single exception of an individual captured in March last (1938) at Dadhar at the entrance of the Bolan Valley—presumably the extreme limit of its distribution towards the west. Most of the specimens were, moreover, collected either in autumn, winter or early spring, and none had been noticed during the months of the summer monsoon (with the exception of one found at Nokh on the 4th June 1935). Hoppers have not as yet been detected anywhere in these areas.

Stray specimens of solitary phase individuals of *Patanga* have been observed in the past at Coimbatore, Nilgiris and a few other localities in South India, and the senior author found a few specimens of the solitary phase of the Bombay Locust collected in the past at Pusa and elsewhere among the locust collections of the Imperial Entomologist at New Delhi. It is, therefore, evident that the area of distribution of this species in India is fairly wide. Outside Indian limits, it is known to occur in Ceylon, China, Sumatra, Java and Borneo [Kirby, 1914], and according to Uvarov [1928] it is distributed all over Southern and South-eastern Asia, and the Malay Archipelago.

According to Lefroy [1906], the locust has only one brood in the year, which occurs during the monsoon season, *i.e.*, between June and September. The swarms breed in open, grass areas in the midst of the forest regions of the Western Ghats, and eggs are preferably laid in soft, clay soils. The adults of the new generation are said to migrate into forests in autumn, remain inactive there during the cold months, and go through a second period of migratory activity in the spring months of March, April and May, flying back to the forests

for breeding by the beginning of the monsoon. It is rather noteworthy that the times of incidence of the *solitaria* forms in the desert areas correspond more or less with the periods of migration noted above, in the case of the *gregaria* swarms. In the absence, however, of fuller information in regard to its distribution in other parts of India, and in view, moreover, of a complete lack of knowledge of the breeding grounds of its solitary phase, it is not possible to appraise the proper significance of the data detailed above.

Data for the Migratory Locust

All available data on *Locusta* have been presented in Table II (Appendix), classified according to the years and seasons of incidence and the regions of occurrence. Information for the earlier years, *viz.*, 1931 to 1935 is quite fragmentary, and much of it has been gathered from notes recorded on the labels of specimens collected during this period. For the years 1936 to 1938, however, fuller information has been available, mainly owing to the observations made by the junior author during this period.

A study of the classified data in Table II would show that, in general specimens of the Migratory Locust are found to be common in the Sind-Rajputana areas only during the monsoon months, while in the Baluchistan region, they are more often met with in the spring season. It is also apparent that breeding occurs generally after receipt of good rainfall, and that few specimens are met with in seasons of drought.

(*a*) Period from 1931 to 1935

Year 1931

No data are available for the Sind-Rajputana region. In the Baluchistan areas, spring rainfall was fairly satisfactory, and Dr. M. Sharif found a few adults in April at Pasni and Ormara, and observed in May, a few hoppers as well as adults at Panjgur. In July, several adults were collected at Sharigh—a locality subject to monsoon rainfall—and in August, a specimen was noted at Dasht Thana near Kolpur (Quetta-Bolan area), and another at Jhatpat on the Sind borders.

Year 1932

In April, a few adults were collected at Quetta and one at Pasni; and in September, a few hoppers and adults were observed in Kolwah (Mekran) in the wake of fairly heavy falls in July. In November an adult was collected at Khairpur in the Bahawalpore State; and near Sodakhor in the Jaisalmer Desert, the senior author observed a few adults along with nineteen specimens of the Desert Locust, in the course of the survey of an open, grassy area at the end of November. The individuals of the Desert Locust were apparently representative of a summer generation bred in north-east Rajputana, which were presumably in the course of their migration towards the west—remarks that might equally apply to the *Locusta* individuals.

Year 1933

A few adults were observed at Akil in west Sind in February, and were probably overwintered individuals. In April, a few adult specimens were found at Pasni. There were fairly heavy spring rains in Mekran in February-April 1933, and since good numbers of *Locusta* adults were observed in the Turbat-Panjgur region in July, it is presumed that breeding had taken place in this region in late spring. As there was good rainfall also during July-August in the Mekran hinterland in 1933, considerable breeding of *Locusta* had presumably taken place in the valleys of the interior, as corroborated by the circumstance that good numbers of adults were collected in Kolwah, Kech, Panjgur and Kalmat during August, September, October and November. A few adults were found in July-August in Kachhi and Lasbela also. In the summer rainfall areas of Sind and Rajputana, a fair number of adults were found in May (Akil), in June (Fort Abbas), and in July (in the areas of west Sind and Bikaner). At the beginning of July 1933, the junior author noted several *Locusta* adults at Shah Hassan in the Manchar Lake region of Sind near the borders of Baluchistan. He was also told that a small swarm of locusts had been seen in this region, and though there was no confirmation of this report, it is not improbable that the species concerned might have been *Locusta migratoria* ph. *solitaria*, flying about in small groups. *Locusta* adults were noted in good numbers between August and December in various places in the Sind-Rajputana area, such as, Bahawalpur, Bhatinda, Thar area, Jaisalmer area, west Sind and Khairpur, and Dera Ghazi Khan and Dera Ismail Khan Districts. As the monsoon was heavy and continuous, there were doubtless two consecutive generations of *Locusta* during the period between July and November in the desert areas.

Year 1934

Drought prevailed in Mekran in spring and no adults were observed in Mekran and Upper Baluchistan between January and June except for a single adult collected in May at Panjgur. In the eastern areas, one specimen was found in April at Mirzapur in west Sind, and in May, a few were found in the Cutch State, and in the Bikaner and Bahawalpur areas. In Mekran, one specimen was collected at Pasni in July. In the Indian Desert areas, the monsoon set in rather early in the latter half of June and continued till the first fortnight of September, but was, on the whole, rather light. In the Thar area of Sind, adults were observed in several places in July, and hoppers were found at Chachro and Rahimki Bazar in August in fields of *bajri* (*Pennisetum typhoideum*). In July both hoppers and adults were sent in from the Ludhiana-Patiala area of Punjab by a correspondent. A few adults were collected also from Sikar (Jaipur State) and Saro (Thar) in September and in October from Dirasar (Bikaner State). An adult was collected at Hingol (Lasbela State) in November 1934.

Year 1935

In February and March, 1935, a thorough search was made at Chachro and Rahimki Bazar respectively for specimens of hibernating locusts among

bushes and under field fences, in localities where breeding had been observed in 1934, but neither *Locusta* nor *Schistocerca* was found at these places, though certain large grasshoppers were found hiding under the fences. This would probably indicate that the individuals of *Locusta* of the new generation produced in July-August 1934 had, as in the case of *Schistocerca*, left the area by the autumn of that year. A single adult collected in March 1935, at Sardarshahr in the Bikaner area in the northern Desert is, however, found in the collections. In spite of heavy winter rainfall in the Mekran, the only records, in regard to the Baluchistan area, are of an adult found in May 1935 at Mand in the Kech Valley, and another collected at Liari in Lasbela State in June 1935. During the summer season, there is only one record of an adult found at Nokh in July for the Rajputana area. The paucity of *Locusta* individuals in both the Baluchistan and Rajputana areas in a year, in which the *Schistocerca* population had increased enormously all over these regions, is rather remarkable. It may, however, be noted that the monsoon did not extend into Mekran, Lasbela and Sind in the summer of 1935, nor was the rainfall in the Desert areas considerable except in July.

(*b*) Period from 1936 to 1938

The extensive observations made by the junior author have served to furnish considerable information in regard to the developments of the Migratory Locust during this period.

Year 1936

There are no records of observations on either adults or hoppers of *Locusta* during the first six months of 1936, in the Baluchistan or the desert areas, except for one specimen noted on the 29th June 1936 at Udasar in Bikaner.

In July 1936, however, adults varying in number from one to one hundred were found in *jowari* (sorghum) fields at Bhag, Gandhawa, etc., in the Kachhi area and at Johi in the west Sind area, indicating that breeding had occurred along the Baluchistan-Sind borders during the spring months of 1936 as a result of favourable rainfall. During August and September 1936, fairly good numbers of *Locusta* were noted during surveys along with individuals of *Schistocerca* in the Thar-Mallani, and Bikaner-Jaisalmer areas. By the 24th of September, however, very few adults of either *Schistocerca* or *Locusta* were noticeable in the Thar-Mallani area, where owing to the development of adverse climatic conditions brought about by the prevalent drought, almost the whole bulk of the new generation of *Schistocerca*, produced during July-August, would seem to have deserted the area. Neither *Schistocerca* nor *Locusta* was seen in this area till June-July 1937.

In the northern areas of the Indian Desert, on the other hand, there was very heavy rainfall (about 15 inches in the course of about ten days) in August 1936, and this led to a considerable and rather protracted breeding of the Desert Locust, which consequently went through a second monsoon generation in this area between September and November. The adults of the second

generation would appear to have overwintered in this area, migrating therefrom, apparently westwards, only by February-March, 1937. No direct observations in regard to the breeding of *Locusta* at this part of the year are on record, but since as many as twenty-two individuals of *Locusta* were found in this area along with specimens of *Schistocerca* during October, November and December 1936, it is not unlikely that *Locusta* had also to a certain extent bred and overwintered in this area.

Year 1937-38

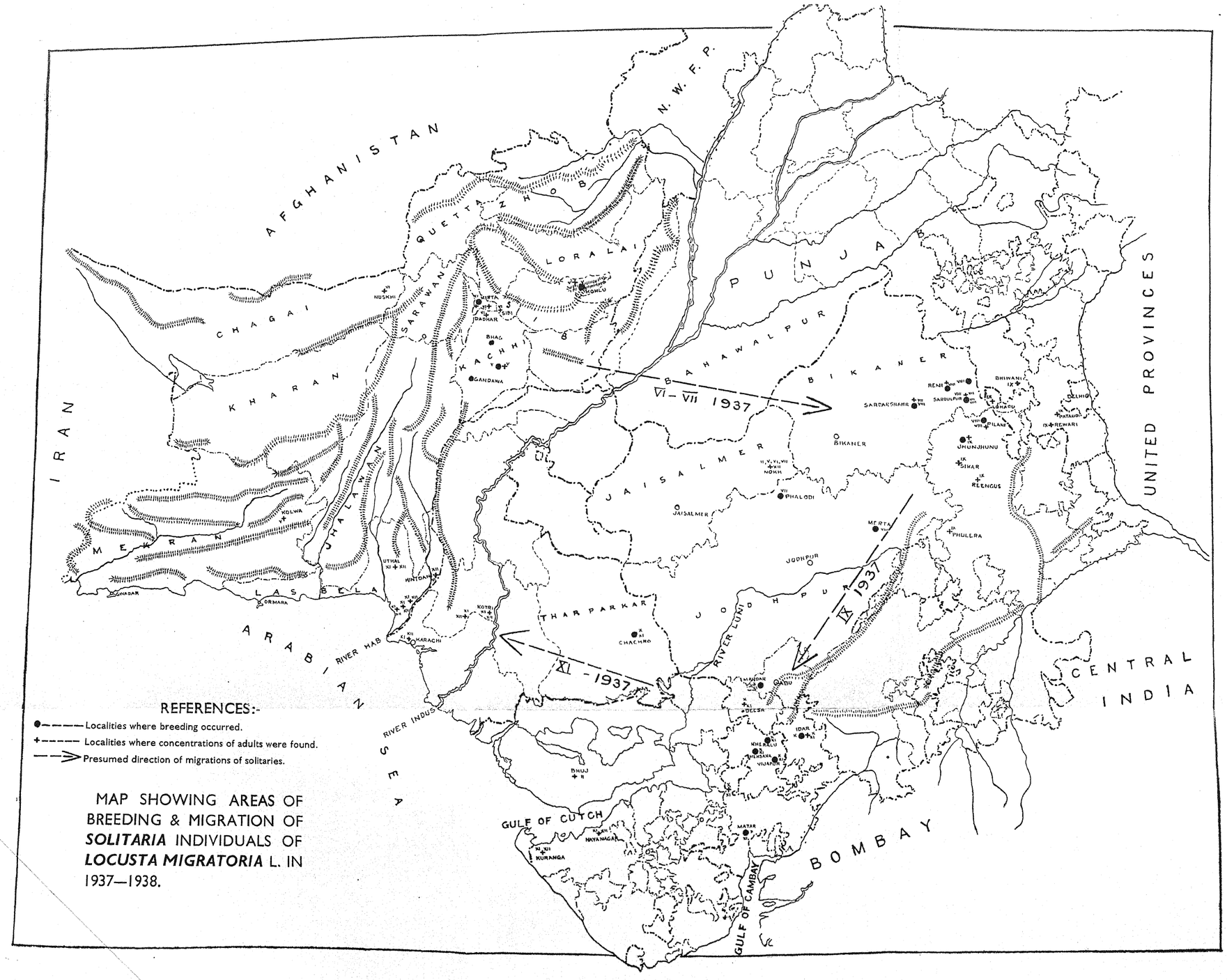
The areas of infestation in 1937-38 have been marked in the map. In the Indian desert areas, there are only three records of captures between January and May, 1937 : one found near Nokh in February, a second near Lathi (east Jaisalmer) in April, and a third at Nokh at the end of May.

1. Spring breeding.—In the Baluchistan area, no observations had been made during the months of January to April 1937, and it was more or less by accident that the junior author visited the Kachhi area during May, when he found both *Schistocerca* and *Locusta* breeding in fair numbers in this area. During June 1937, he found fairly good numbers of *Locusta* adults at Sibi, Dadhar and Mashkhaf. At Kirtha in the Bolan Valley, he found quite an intensive breeding of *Locusta* in June, among cultivated fields of *jowari* and water-melons, and among grasses in the vicinity. The density of population was estimated at over one million per square mile, and he could actually count seventy-seven *Locusta* adults and twenty-eight hoppers within a small portion—180 square yards in extent—of one of the fields. (It may be mentioned in this connection, that when the same area was re-visited in August 1937, a few adults only were found.)

A report accompanied by specimens received from the Tahsildar of Kohlu (a division of Sibi District in Baluchistan) indicated that hoppers and adults of *Locusta migratoria* as well as of certain grasshoppers (mainly *Oedaleus*), had been found in considerable numbers in the Kohlu valley during May-June 1937. Owing to the favourable distribution of rainfall in February, March and April (Table III—Appendix), it is quite probable that similar breeding of *Locusta* had extended into various other neighbouring hill-valleys of Baluchistan, such as those of Loralai and Sibi Districts and of the Marri-Bugti country.

During May, the Mekran Survey Assistant reported the presence of *Locusta* adults in the Kolwa valley of Mekran, and on the 29th June, one of the Survey Fieldmen found five *Locusta* adults at Nuskhi in Chagai District.

2. Summer brood.—In the desert area, very few specimens of *Locusta* were found in the desert along with *Schistocerca* during surveys made in June at the Nokh Outpost, whereas during July, as many as fifty individuals of *Locusta* were collected in association with an equal number of *Schistocerca* between the 1st and the 25th July, but from the 26th July both species became scarce at Nokh. At Phalodi, thirty-two miles south of Nokh, where a heavy fall of six inches of rain had been received on the 1st July, good numbers of *Locusta* hoppers were found on the 31st July in a grass-covered depression in the midst



REFERENCES:-

●———— Localities where breeding occurred.

+———— Localities where concentrations of adults were found.

——→ Presumed direction of migrations of solitaries.

MAP SHOWING AREAS OF BREEDING & MIGRATION OF ***SOLITARIA*** INDIVIDUALS OF ***LOCUSTA MIGRATORIA*** L. IN 1937—1938.

FIG. 1. *Jowari* (sorghum) field severely defoliated by hoppers of *Locusta migratoria* L. at Moti Dau, Mehsana District, Baroda State (November 1937)

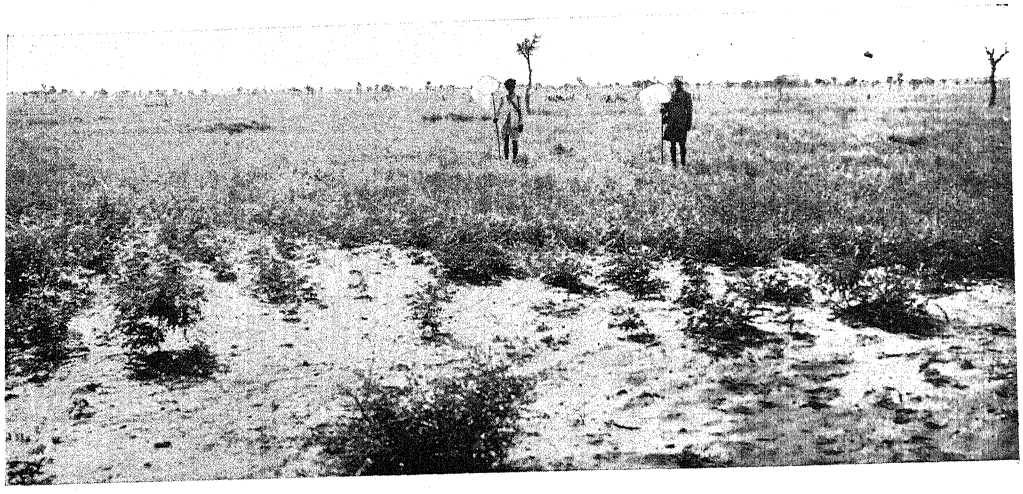


FIG. 2. Low-lying area covered with *Tephrosia* scrub and grasses at Sadulpur, Bikaner State, where large concentrations of *Locusta migratoria* ph. *solitaria* were seen on 10th July, 1937

of sand dunes, while small numbers of *Schistocerca* hoppers were found among bushes on the sides of the dunes. In the Thar-Mallani area, in the southern desert, small numbers of *Locusta* were found appearing in July 1937 along with individuals of the Desert Locust. Similarly, in the vicinity of Karachi too, both *Locusta* and *Schistocerca* were found occurring together in July, 1937, during local surveys.

In the East Bikaner area, neither *Locusta* nor *Schistocerca* adults were noted during a tour in the middle of May in the Nohar-Reni-Sadulpur section, but by July, fairly large concentrations of *Locusta* and somewhat smaller groups of *Schistocerca* were found at Nohar (3rd July) and at Sadulpur (9th and 10th July). In the latter place especially, the *Locusta* population, estimated at about 80,000 per square mile, occupied a very large extent of rather low-lying area, covered with a heavy growth of *Tephrosia* scrub and grasses (Plate V, fig. 2), while the Desert Locust concentrations (about 10,000 per square mile) were found on sand-mounds in slightly higher and drier situations.

On the other hand, few *Locusta* adults were noticeable in the Kachhi-Bolan area during July-August.

With the heavy rainfall received during the latter half of June and in July, *Locusta* hoppers were noticeable in greater or smaller numbers almost all over the desert regions, except in the Jaisalmer area. Specially large numbers of hoppers were noted along the eastern borders of the Desert as in the Reni-Sadulpur area of Bikaner, and the Shekawati area of Jaipur. The junior author, for instance, in the course of his examination of the Sadulpur neighbourhood, met with on the 11th August 1937 very large concentrations of hoppers and adults in certain places. By actually counting the number of hoppers and adults in measured plots of ground, he estimated the density of the *Locusta* population to be between one and three millions per square mile in the case of hoppers, and between two and four millions in the case of adults. In regard to the general colouration of the hoppers, he observed that it was mostly greenish, or green and brown, though in a certain number of them, he found the green replaced by reddish brown on the ventral surface, the reddish tinge being found extending also in some cases to the legs, the post-ocular streak and the pronotal stripe. He also found several other hoppers which had blackish brown dots interspersed on a background of grey brown and a few others which were of coal-black colour.

Similar dense concentrations of hoppers and adults were noted in August in the Jhunjhunu-Pilani area of the Jaipur State.

In most parts of the Indian Desert, there was a cessation of monsoon rainfall from the end of July till the 7th of September (Table III—Appendix), and as a result thereof conditions of drought prevailed, which began to affect cultivated fields as well as the natural vegetation, causing them to dry up. As high temperatures (for instance a maximum of 105°F. at Bikaner early in September) and comparatively low humidity prevailed during this period, it is surmised that the ecological conditions in these areas became uncongenial to adult locusts, and created in them an urge to abandon these

areas. As several dust or thunder storms passed over this area during the last week of August in the afternoons, the more mature forms of both *Schistocerca* and *Locusta* would appear to have migrated along with the storms out of the east Bikaner area. The decrease in numbers of the locusts was so evident that it had attracted the notice of the cultivators of the Sadulpur area, who declared, when enquiries were made during a visit at the beginning of September 1937, that '*phidkala*'—a local term denoting locust and grasshopper infestation—had considerably diminished in their fields for the past ten days. During September, fair numbers of *Locusta* adults, however, still persisted in the eastern desert areas, and small numbers were found in some places along the fringes of the desert, *e.g.*, Loharu, Bhiwani, Pataudi Road, Rewari, Reengus, Sikar, Jhunjhunu and Phulera, though, by October-November, very few *Locusta* adults were to be seen at the very places where they had been so abundant in August.

3. October infestation.—During October 1937, two reports of rather severe damage to rain-fed cereal crops were received—one from the Sirohi State and the other from the Mehsana District of the Baroda State. Both these areas were visited, the former by the junior and the latter by the senior author, and investigations were made. It was reported that flying swarms had not been seen anywhere, but that large numbers of hoppers, which had at first been mistaken for the young ones of ordinary grasshoppers (locally known as *khappadi*), were found damaging the crops in October. As a result of enquiries made, it was elicited that even old ryots (cultivators) could not recollect having seen a similar type of damage ever before, the attacks of the Desert Locust swarms fairly common in the Gujarat area being said to be altogether of a different character.

As a result of official enquiries made in the States adjoining Sirohi and Mehsana areas, and of a personal visit paid by the junior author to various localities in Kathiawar and Gujarat in December, 1937, it was found that the following localities had, in addition, been similarly infested at about the same period: *1. Palanpur State*: Some damage to *bajri* and *jowari* fields at Kuchawada village of Deesa Tahsil, and light damage to *jowari* at Umardeshi and Palanpur; *2. Idar State*: Paddy and *jowari* damaged in the villages of Panpur, Lalpur and Purbada in the western part of the State; altogether 120 acres of paddy and 128 acres of *jowari* were reported to have been damaged; *3. Kaira District, Gujarat*: A small block of rice fields in the Matar Taluka was reported to have been attacked in November. As regards the Sirohi and Mehsana areas, the following information was gathered in regard to the extent of damage: *Sirohi State*: Several villages in the Pamera and Mandhar Tahsils (mostly sand-loam areas) in the south-western part of the State were affected, of which four villages were badly damaged. *Bajri* (*Pennisetum typhoideum*) and *barti* (*Panicum miliaceum*) were more or less defoliated and the ears damaged, and many fields were said to have been prematurely harvested to save them from attack. Grass was also attacked. *Mehsana District*: The affected areas were situated in the Talukas of Mehsana, Kheralu and Vijapur, and were thus all located in the north-eastern quadrant of the Mehsana

District. The total area attacked was probably over 300 acres of cultivation which consisted mostly of *jowari*, and to a certain extent paddy, sugarcane and *bajri*. The worst attacked locality was Gundrasan, where several *jowari* fields were absolutely stripped of leaves (Plate V, fig. 1).

From the data available, the origin of the infestation may perhaps be traced as follows. There was good rainfall in the Sirohi and Gujarat areas during July, which was favourable for the early crops, such as *bajri*, *jowari* and pulses. Then followed a long drought in August, which was broken only on the 8th September, when a depression, originating in the Bay of Bengal and travelling north-east to south-west, ushered in heavy rainfall in Gujarat and Rajputana and it is surmised that a good proportion of the large *Locusta* population known to have been present in the areas of Eastern Rajputana were carried by the storms associated with the depression, into the areas of south-east Marwar, Sirohi, Idar, Palanpur and Mehsana. The transference of locust population would appear to have occurred not in the form of swarms, but as large groups of individuals. It is rather interesting to note that the regions infested, *viz.*, south-western Sirohi, Palanpur, Idar, and the north-eastern parts of Mehsana, form an area of undulating sandy loam country, 300 to 400 ft. above seal-level, abutting on the bases of the Abu and Aravalli ranges. The surface soil layers of this area are composed mostly of a thick mantle of fine sandy loam (or perhaps loess) deposited in the form of a succession of low dunes by aeolian action in a former drier age. The geographical position of the infested areas rather suggests that the body of migrating locusts, on emerging from the hilly tracts of S. E. Rajputana, lying to the north-east of the plains of Gujarat, had selected the nearest wet patches along the fringes of the sandy country for depositing their eggs. *Locusta migratoria* has restricted food-habits, and as a rule attacks only plants of the grass family. In Sirohi, it was found to attack *bajri* and *barti* (*Panicum miliaceum*), and in Gujarat, *jowari* (sorghum), sugarcane and rice. In addition, various wild grasses were also devoured. It is probable that the *Locusta* adults had, on arrival early in September, concentrated in fields of *bajri* and *jowari*, and as they were presumably already mature, they were able, almost immediately, to deposit eggs in the more moist situations among cultivation. Hoppers had probably emerged by the end of September and the beginning of October and begun to feed on the standing crops of *bajri*, *barti*, *jowari*, sugarcane and paddy before they were noticed and reported. Many of the hoppers had become adults by the end of October, and by November 10th, most of the adults had already disappeared from the scene of their activity. Though a fair number of bird or bat predators were noticeable, the diminution in numbers was so sudden that it could not have been solely due to the activity of natural enemies. Stray hoppers were, however, being found in these areas even in December, which would indicate that stray egg-laying had continued over a long period.

4. Second brood of Locusta *in the desert area.*—The fall of good rain in September, in the southern areas of the desert, led to the development of a second brood of *Locusta* in the Thar area, hoppers being found in fair numbers throughout October and the first week of November. Hoppers were similarly

observed in the Sanchore-Raniwara area adjoining the Sirohi area in November. At Chachro a sudden decrease in the population of *Locusta* adults was noticeable after the 10th November, and by the end of December, few specimens of *Locusta* were to be met with there.

We are indebted to Dr. Keshodas Baweja of Lyallpur for the information given by him in regard to the presence of fairly large numbers of *Locusta* adults in October on *sarkanda* (wild *Saccharum*) at Campbellpur (Attock District) in the Punjab, and since Dr. W. Burns, Agricultural Expert, Simla also found a few adults in September at Simla, it is possible that *Locusta* had also been breeding in various suitable grassy areas in Punjab during the monsoon period.

5. Distribution of Locusta *during November, December and January.*—During the second week of November 1937, one of the survey Fieldmen found fairly good numbers of *Locusta* adults in *jowari* fields between Jhimpir and Thano Bulakhan, and about the same time, fairly large numbers were similarly found in *jowari* along the banks of the Hab River near Karachi. On the 22nd November, and subsequent days, quite large numbers of *Locusta* adults were noted in grassy areas situated between Naka Kharrari and Lak Baduk, the southernmost part of the Ambagh Reks, the density of population being estimated to be between ten and twenty thousand per square mile. These reks had been under observation since 1933, and never before had such large numbers of *Locusta* been noticed on them. One of the survey Fieldmen, who had been examining the Hinidan area in the interior of Lasbela State, found on the 15th November a fairly large concentration of *Locusta* adults (density about 20,000 per square mile), in *jowari* fields at Hasan Pir, and he was assured by the cultivators that these locusts had been noticed on the crops only recently. He met with similar concentrations on crops in several other places, also alongside the Sind-Lasbela borders, such as Thana Kila, Hinidan, Koh Mahir, Pabuni, and Goth Sherkhan. Subsequently, fair numbers of *Locusta* were also found in the interior of the Lasbela State, at Uthal, Wahyara, Thana Tappi, Nakhetri, and Liari, and one specimen, as far west as Chandragup. A few stray specimens were also observed in the Ormara and Kulanch areas in November. In December, fair numbers of *Locusta* were found at Meting (Karachi District) on the *jowari* crop.

During a rapid survey tour made in December, 1937, in the Gujarat-Kathiawar areas and again in February, 1938 in the Kathiawar-Cutch areas, small concentrations of *Locusta* adults were noticed in December at Nawanagar and Kuranga along the west coast of Kathiawar, as well as stray specimens of adults and hoppers in parts of Kathiawar. In February, 1938, a few adults were found in the Junagarh area of Kathiawar, and small numbers at Bhuj, Bhachau, Mandvi, etc., in the Cutch State. Locust droppings were also found in wheat fields and grass areas in many localities in Cutch and Kathiawar indicating that locusts had visited them some time ago.

6. Distribution in spring, 1938.—It was noticed that the large numbers of *Locusta* adults found in *jowari* fields in Karachi-Lasbela areas, disappeared after the crop-harvest, and it is presumed that they became scattered over the

area. In February, stray hoppers were seen in many parts of the Lasbela State, and it is surmised that egg-laying had taken place after the fall of good winter rains in December and January last, but few adults were noticeable. No locusts were observed in December in the Kachhi area, whereas during a visit paid to the Kachhi, Bolan and Harnai areas of Upper Baluchistan in March 1938, it was noticed that *Locusta* adults were present there in varying numbers concentrated on the irrigated wheat crop, which was then mostly in ears. As many of the *Locusta* females showed traces of soil and froth on their valves, there is little doubt that they had already laid eggs in the soft, moist loamy soils of the wheat fields. As by the time wheat is harvested, neighbouring fields would have been sown with *jowar* and melons, the hoppers will presumably transfer themselves to the latter on hatching. It is obvious that the large concentrations of hoppers noted last year in June at Kirtha should have been the result of similar concentrated egg-laying in wheat fields in March last. (Large numbers of hoppers have since been reported to have been found at Kirtha in May 1938).

Analysis of the data and inferences

In analysing the data of the past seven years—as detailed above—the following observations may perhaps be made.

1. In the *Sind-Rajputana* area, which is subject to monsoon or summer rainfall, the solitary phase individuals of the Migratory Locust, as in the case of the Desert Locust, are most in evidence during the monsoon months, and are least noticeable during the dry season between January and May. *Locusta* breeds in this region soon after the fall of monsoon rains, and the adults of the new generation generally make their appearance during August or by the beginning of September. If the rainfall continues to be satisfactory in August and September, a second generation may be produced in the desert during October. On the other hand, in the Baluchistan areas, *Locusta* is generally to be seen in the spring months, and breeding may take place in spring or early summer. As the eastern parts of Baluchistan, such as Loralai and Sibi Districts, Lasbela, Jhalawan, and Kachhi, and the Kolwa and Panjgur areas of Mekran, generally come under the influence of the monsoon to a greater or lesser extent, a summer brood of *Locusta* may come into existence in these areas, as in the case of *Schistocerca*. Should there be no rainfall in summer, neither adults nor hoppers of *Locusta* are likely to be found during the summer months in Baluchistan.

2. During the year 1936, the effect of climatic fluctuations on the populations of the Desert Locust was under study during September and October in the desert areas. It was noticed that the populations of *Schistocerca* as well as of *Locusta* rather suddenly disappeared in the southern areas during the latter half of September and the main factors concerned in this phenomenon were found to be a rise of diurnal temperatures, a fall of relative humidity of the atmosphere, and the appearance of dust-storms in the afternoons. It is conjectured that the above-stated changes in the climatic conditions had rendered the environment in the desert uncongenial to the adult locusts and

had caused them to take to flight and leave the area, the time of flight synchronising with that of the occurrence of the storms. On the other hand, by November there was a definite fall in the temperature levels as well as a slight rise in atmospheric humidity due to the advent of the cold weather, and in areas, like that of Nokh in 1936, where locusts had become fledged late in the season, there was no attempt on their part to leave the area. They passed the winter there, and began to disappear only at the end of February 1937, when dry hot conditions again began to prevail.

An emigration of adult locusts was brought about in August-September 1937, when on account of the prolonged break in the monsoon in August 1937, conditions of comparatively high temperatures and low humidity prevailed in the north-eastern areas of the desert, as had happened in September 1936 in the Thar Area.

3. Though various observations made during the period 1931-1935 rather vaguely pointed to the possibility of a shifting of the *Locusta* population from the spring rainfall areas of Baluchistan into the summer rain regions of Rajputana and *vice versa* with the change of the seasons, no definite evidence was forthcoming. During the period 1936-1938, however, owing to the occurrence of heavy as well as extensive breeding of *Locusta* both in spring and summer in different parts of North-West India, fairly definite indications of a transfer of population from one area to another have become available (*vide* map appended). First, fairly good numbers of *Locusta* adults were noticeable in the Jaisalmer area during the months of October-December 1936, but were not to be seen there from January 1937 onwards till June next. Secondly, heavy breeding was noticed in May and June 1937, in the Kachhi area and in the hill-valleys of Baluchistan, such as Bolan and Kohlu, bringing about a high rise in the density of population. By June-July, however, there was a very considerable decrease in numbers here. Thirdly, at about the same time, heavy concentrations of *Locusta* adults made their appearance in the eastern parts of the Indian Desert, as at Reni and Sadulpur. There was extensive breeding in these parts in July-August, but by September the density of population had greatly decreased, evidently by emigration. Fourthly, in September-October an incursion of *Locusta migratoria*, followed by a heavy infestation of cereal crops, had occurred in the Sirohi-Idar-Mehsana areas, and comparatively light breeding in the Thar area; but during November, the adults produced here were found disappearing. Fifthly, concentrations of *Locusta* adults of varying densities were observed on *jowari* and wheat crops during November-December in the Karachi-Lasbela areas, as also, in parts of Kathiawar and Cutch; and lastly, very few *Locusta* adults were noticeable in February 1938, in the Karachi-Lasbela areas, whereas, fairly good numbers were seen in wheat crops in March in the Kachhi-Bolan-Harnai areas of Baluchistan.

4. So far, no direct evidence has been obtained in regard to the ability of the solitary phase form of *Locusta* to migrate from place to place. Marking experiments could not, unfortunately, be tried early enough during the year, and out of about sixty specimens marked and released in December 1937, at

Ambagh, none have so far been recovered. As yet, no actually observed cases of high flights have been recorded, except, perhaps, in one rather peculiar instance, in which a *solitaria* individual of *Locusta* was observed, on the 1st August 1937, dropping like a stone from above at about 8 P.M. at night, attracted to the bright light of an incandescent mantle lamp kept in the courtyard of the Nokh Outpost. As the courtyard in question was shut off by fairly tall huts on three sides and was open only to the north-east, and as a strong south-west wind was blowing at the time, it was surmised that the locust had been attracted to the light while migrating high above ground-level with the prevailing wind. On that day, it may be added, neither *Locusta* nor *Schistocerca* was located anywhere in the vicinity of Nokh during survey.

On the other hand, there is strong indirect evidence in regard to the reality of migration. The very fact that in certain cases, large numbers of locusts had often been met with in places where none had been seen a short while ago, and in certain others, few were noticeable in localities where they were known to have been present some time earlier in very large numbers, may be taken as presumptive evidence in regard to their powers of migration. The rather extraordinary sequence of occurrences chronicled last year (1937) (see map attached), *viz.*, the breeding in May-June in Kachhi and Bolan, and then in July-August in east Bikaner, followed by the reported infestation in Sirohi and Gujarat in September-October, and the concentrations observed in crops in Lasbela and Kathiawar in November-December, and in Kachhi-Bolan in March, 1938, would really appear to be an organically linked chain of events, that cannot be explained except on the basis of a hypothesis of a migration flight of individuals from one breeding ground to another under the directive force of the prevailing winds.

5. Although the *solitarias* of both *Locusta* and *Schistocerca* show a good deal of agreement in their general breeding habits and in their reaction to climatic conditions, they present certain striking contrasts in their ecological preferences, which are of importance in a study of the factors that bring about swarming. Whereas the Desert Locust favours comparatively dry situations, prefers sandy areas for egg-laying, and is, on the whole, of cosmopolitan tastes, though usually exhibiting a definite choice for certain wild food-plants, *Locusta* has a preference for relatively more moist micro-climates, generally selects loamy soils for oviposition and has a strong predilection for food plants of the grass family, whether wild or cultivated. In the areas of the Indian Desert, *Locusta* generally lays eggs in clayey hollows in the midst of sand-mounds, and the adults also prefer to concentrate on densely grown crops, such as *bajri*, *jowari*, wheat or millets, or among thick clumps of grasses. In this connection, Faure's remarks [1932] on the habits of *Locusta m. migratorioides* ph. *solitaria* in South Africa are of much interest: " The habits of this locust stand in direct contrast to those of *Locustana pardalina* ; the latter is a lover of open spaces, while the former hides in the dense growth of lucerne, and is exceedingly difficult to catch, mainly because it gets down near to the ground and hides. It has been found in greater numbers in weedy (*i.e.*, grassy) fields of lucerne. At Brandvlei, I found this locust in the stubble of recently

cut wheat lands. In De Aar district it was taken in maize lands—small irrigated patches in the semi-arid Karroo—but not in the open veld where *L. pardalina* occurs".

6. The fact that this locust tends to concentrate in patches of cultivated cereals and to lay eggs therein, if the conditions are favourable, is important from the point of view of the formation of incipient swarms. In the Bolan valley, as also in similar other hill-valleys of Baluchistan, arable land is limited to patches of fine silt of varying extent, deposited at the bottom of hollows between rocky, barren hill ranges, and the extent of cultivation of wheat, and later on of *jowari* is dependent on the quantity of spring or stream water available for irrigation. By the time the hoppers emerge from eggs laid in the wheat fields, the crop is ready to be cut, but since the neighbouring fields are generally sown with sorghum and melons, they may feed either on grasses in the harvested fields or transfer themselves to the sorghum plants. There are, thus, great possibilities of concentrated breeding of *Locusta* taking place in the narrow hill valleys of Baluchistan. In the high dune areas of the Indian desert, *bajri* (*Pennisetum typhoideum*) is generally cultivated in the hollows between the sand-ridges, where the soil is comparatively more clayey and moist, for which reason, *Locusta* also selects such areas for egg-laying. If the number of migrating locusts should be large, crowded oviposition might result, but in seasons of good rainfall, the general vegetation, including various grass annuals, springs up in such profusion on the vast expanses of the desert, that the hoppers would tend to get dispersed and isolated amongst the dense vegetation, unless the population is very dense.

Notes on the phase characteristics of the *Locusta* population in 1937

In the course of the year 1937, a collection of *Locusta* adults was made from the different areas where breeding of *Locusta* or the appearance of adult individuals or concentrations had been noticed. These collections were studied by Mr. R. L. Gupta, Biometrical Assistant, attention being concentrated mostly on the determination of the Elytron/Femur ratios, the results of which have been classified in Table IV (Appendix). Zolotarevsky [1933] had, in his studies of *Locusta m. capito*, tentatively fixed the limits for *gregaria* ratios for *Locusta* at 2·00, so that those showing 2·00 or above were to be considered as *gregaria* and those below as *solitaria*. From a study of the *solitaria* specimens collected in India, it would appear that the limit for *solitaria* should be fixed somewhat lower, as most of the measurements lie between 1·80 and 1·90. For the sake of convenience, 1·90 has been taken as an arbitrary upper limit for *solitaria*, and ratios between 1·91 and 2·00 have been classified in this paper as representing '*intermediate*' or *transiens* ratios, and those above 2·00 as *gregaria*.

Of the forms collected in Sibi, Kachhi and Bolan areas in May and June only 14 per cent showed the '*intermediate*' ratios, *i.e.*, E/F ratios of over 1·90. The great majority were all '*solitaria*' in character.

The collection of adults made in June-July in the Jaisalmer area and the forms found in the concentrations found in July in east Bikaner area had a higher proportion of the ' intermediate ' ratios, *viz.*, about 32 per cent.

Among the forms found in Thar-Mallani area in July-August, and in the collections made in the Bikaner-Jaipur-Jodhpur areas in September-October, the proportion of ' intermediate ' ratios was low (6 to 9 per cent), and in the Thar-Mallani area in November, the proportion is higher (25 per cent).

In the areas of infestation in Sirohi, examined on the 31st October 1937, it is interesting to note that 31 per cent of the number of specimens collected showed ' *intermediate* ' ratios, 46 per cent ' *gregaria* ' ratios, and the rest, about 23 per cent, ' *solitaria* ' ratios. In the Mehsana area of infestation, a collection of specimens was made only by the second week of November, at a stage when a great many of the adults of the new generation were reported to have already disappeared ; *gregaria* forms were not found in this population, but about 75 per cent of the specimens collected showed ' *intermediate* ' ratios.

Finally, the concentrations of adults found in sorghum fields in November-December, in the Lasbela—Karachi areas, had about 23 per cent of ' *intermediate* ' forms, the rest being all *solitaria.*

The above results show that, during 1937, the proportion of ' *intermediate* ' to ' *solitaria* ' showed a general tendency to rise with the gradual rise of population. The degree of development of the higher phase ratios would appear to be dependent on the strength of concentration at the breeding period, the *gregaria* forms having appeared this year only at the peak of the breeding.

Specimens of ' *transiens* ' and ' *gregaria* ' forms collected in the Sirohi area were sent to the Imperial Institute of Entomology, London, and the phase determinations were confirmed. Dr. Uvarov, who examined them, stated that in the absence of a larger series of *gregaria* forms, it would be difficult to say which sub-species of *L. migratoria* the Indian forms are to be referred to. He, however, thought that the Indian forms approached the form *manilensis* in their size, though in the shape of the prothorax they approximated *migratorioides.*

Natural enemies observed

The natural enemies observed so far include some of the common bird predators, a bat and two mite parasites.

The birds found attacking this species were : 1. the Indian Roller (*Coracias*), the Bee-eater (*Merops viridis*), the Grey Shrike (*Lanius*), the Mynah (*Acridotheres*), the Drongo (*Dicrurus*) and the Starling (*Pastor roseus*), the last being found occurring in large flights.

In one of the places in the Vijapur Taluka of Mehsana District, a fairly decent heap of cut wings and hind-legs of *Locusta* was discovered at the base

of a large hollow in the trunk of an old *mahua* (*Bassia*) tree in the vicinity of affected fields, indicating that bats had been actively feeding on *Locusta* adults at night.

Of the mite parasites, one was the common red mite found on the wings of various locusts and grasshoppers, possibly the young ones of *Trombidium grandissimum*. The other—possibly a Tarsonemid—is a mite of cream yellow colour, found occurring in masses on the thorax, abdomen and wings of the adult. Eggs, larvæ and adults of the mite generally occur crowded together on the locust, and at times the percentage of infested locusts is very high.

Past records of swarming of *Locusta migratoria* in India

Most of the known records of locust swarms in India refer either to *Schistocerca* or *Patanga*, and there is no definite record of swarms of *Locusta*, although the *solitaria* form of *Locusta* is known to occur throughout India.

There is, however, one striking case of infestation, which Cotes [1891] has recorded referring to it as the Madras Locust Invasion of 1878. According to him, locusts invaded the whole of the Madras Presidency in 1878, the last year of the great South Indian Famine, causing damage high enough to aggravate the distress resulting from the famine. "The young locusts (hoppers) began to appear in January and were found in great numbers in different districts from that date on till September and October—the earliest swarms being found in the south and west of the Presidency and the later ones in the north and east. The winged locusts were first observed in the end of March and the beginning of April in the south-west (Wynaad and Nilgiris), and they afterwards spread over the Presidency to the east and north, not finally disappearing till about November and December. Young locusts (hoppers) which were found in the early part of May in the Udamalpet taluq were supposed to be the offspring of the large flights of winged locusts that had appeared in the preceding February in the same taluq, and it is stated that locusts were observed pairing in the latter part of June in the Salem District (situated towards the north-east)". The visitation was apparently of considerable magnitude, since control measures such as the destruction of large numbers of hoppers by driving them into lines of burning straw, and driving winged locusts from one village to another by beating through the crops, had to be adopted.

Specimens supposed to represent the Madras Locust preserved in the Central Museum, Madras, are said to have included no less than six distinct species of Acrididæ, such as *Cyrtacanthacris*, *Acrida*, etc., of which *Pachytylus cinerascens* (*Locusta migratoria danica*) was the only representative of a real locust.

It may be observed that the months of January, February and March are warm enough, in the parts of South India referred to by Cotes, to favour locust breeding. The prevalent winds in these regions are from north-east to south-west during the period between October and February, and south-west to north-east between May and September, so that the direction of the

locust movements recorded coincide with the wind directions. Though from the data available * there is no definite indication as to the identity of the locust concerned, it appears probable that the species in question was *Locusta migratoria*, and the origin of the infestation was, possibly, derivable from some concentrated breeding that had taken place in grass areas near the foot-hills of the Western Ghats Ranges during the rains of the north-east monsoon.

Possibilities of *Locusta* forming swarms in India

Locusta migratoria ph. *solitaria* appears to resemble the solitary phase of *Schistocerca gregaria* very closely in the broad outlines of its life-history and habits. In both, there is no diapause in the egg-stage; under natural conditions, oviposition is, in both, dependent on the occurrence of rainfall; the period of duration of the egg and hopper stages is short whenever conditions of temperature and humidity are favourable; and in both species, there is the possibility of the occurrence of two or more generations during a year, in case the individuals can reach appropriate rain-belts at the right time. Although the *solitaria* form of *Locusta* is known to occur throughout India, no continuous observations have, as yet, been made as to its annual life-cycle in India, except in the desert areas of north-west India.

It is, however, evident that the possibility of the continuance of an infestation over a series of years of a locust possessing the life-habits of *Schistocerca* or *Locusta* would be dependent solely on its ability to reach suitable areas of rainfall, so as to ensure the development of at least two annual broods in order that its numerical strength might be maintained. In the major part of the Indian continent, rains are confined to the period of the summer monsoon between June and September, and the rest of the year is on the whole comparatively dry. On the other hand, the southern parts of the Indian Peninsula are under the influence of the retreating or north-east monsoon which lasts between October and January. Again, the north-western parts of India, including Baluchistan, North-West Frontier, Punjab, Kashmir and part of the United Provinces, receive varying amounts of winter rainfall distributed in the period between December and March, in the course of the development of a series of western disturbances which originate on the Mediterranean coast and pass eastwards across Iraq and Iran. Both in the case of South India (with the exception of the West Coast) and Baluchistan (excepting the eastern parts), rainfall is comparatively light during the monsoon period.

In north-west India, the occurrence of a spring brood in Baluchistan, followed by a summer brood in the Rajputana desert area, is rendered possible by the differences in the time of occurrence of rainfall in these regions and the active part taken by the seasonal winds in the transfer of the locust population from one region to the other during summer and autumn. On the other

* Through the courtesy of the Development Department of the Government of Madras, the Proceedings of the Board of Revenue, Madras for 1878, containing the accounts of the 1878 infestation, have since been studied, from which it has now become fairly evident that the species concerned was *Locusta migratoria*.

hand, the winter rain area of South India would appear to be cut off from the breeding areas in the desert not only by the great distance but also by the lack of favourable wind agency. If, for example, the seasonal winds during November, 1937, had been such as to transport the high population of *Locusta* produced in the Sirohi-Mehsana areas, into the north-east monsoon areas of Madras, there should have been the danger of a serious infestation developing in South India.

Even in the Baluchistan areas, winter rainfall is late and generally scanty, and at any rate, owing to the temperature conditions of winter, breeding would not be possible in the hill-valleys of Baluchistan till March-April. Should there be heavy spring rainfall in Baluchistan, followed by concentrated breeding, and should there be two successive generations in the monsoon areas, as had happened in 1937, there might be a danger of swarming of *Locusta* in India, but in the present state of our knowledge, it is not possible to make any statement as to whether the swarms of *Locusta*, when once they are started, would be able to continue their activity in the rest of India, on the analogy of the behavior of *Locusta m. migratorioides* in Africa.

Significance of the phenomenon of migration among the *solitaria* locust population

The significance of the powers of migration shown this year by the *solitaria* population of *Locusta migratoria* in north-west India cannot be over-estimated, especially with reference to the formation of incipient swarms. For instance, during the investigation of the serious infestations reported from the Sirohi and Mehsana areas in 1937, the outbreaks should have had to be considered as having been of purely local origin, if the facts in regard to intensive breeding in the Baluchistan and Bikaner areas had not been known. Similarly, the disappearance of the forms of the new generation should have otherwise been attributed to the action of natural causes, such as the activities of natural enemies. With the fairly definite circumstantial evidence obtained now in regard to the migration of the *solitaria* individuals, we have now clues of value to determine whether breeding of any considerable magnitude could be expected at any particular time in north-west India.

Though it is risky to hazard any statement with reference to regions of which one has no personal acquaintance, the senior author of this paper is inclined to believe that it is not very unlikely that the solitaries of other races of *Locusta migratoria* in Africa, Europe and China may also be found to have similar habits of migration.

In regard to the study of the factors which contributed to the development of the primary outbreak centre of *Locusta m. migratorioides* R. and F. in the current outbreak in Africa, which has achieved a phenomenal spread covering over ten million square miles, the senior writer of this paper would venture to make a few observations. The very interesting analysis made by Lean [1931] in regard to the movements of the early swarms of *Locusta m. migratorioides* in the regions of West Africa during the period 1928—1930

would indicate that the movements have been, in general, north-east to south-west in the autumn and south-west to north-east in early summer, coincident with the prevailing direction of the seasonal winds of the period. Breeding would appear to have occurred on the west coast in Senegal, French or Portuguese Guinea in April after the early rains; and the new generation of locusts would appear to have moved eastwards in June-July into the Niger area, where breeding would seem also to have taken place thereafter. In autumn the swarms of the new generation would appear to have been moving westwards once again. It was only in 1930, between May and July, that a considerable part of the swarms moved further east into Anglo-Egyptian Sudan, from which area the succeeding generation flew southwards, thus marking the commencement of the main invasion of the central parts of the African Continent. It may be noted here, however, that in spite of the great developments in the central and southern regions of Africa during the period 1931 to 1936, West Africa has continued to be subject to the periodical invasions of swarms, the direction of movements of which would appear to have varied more or less directly with the trend of the prevailing winds at the change of the seasons [Uvarov, 1933; 1934; 1935; 1936]. Observations on the occurrence of seasonal migrations of *solitaria* individuals of *Locusta migratoria* in this important area would be of particular interest.

Summary

1. In the course of periodical surveys carried out since 1931 in the desert areas of Sind, Baluchistan and Rajputana, in connection with the observations on the seasonal distribution of the solitary phase of the Desert Locust, stray specimens of the solitary phase of the Bombay Locust (*Patanga succincta*) and the Migratory Locust (*Locusta migratoria*) were being found, and in view of the evidence obtained in 1935 as to the capability of the *solitaria* individuals of the Desert Locust of making long, seasonal migrations from winter-rain areas to summer-rainfall areas in early summer, and back again in autumn, all the data gathered during the past seven years in regard to the Migratory and Bombay Locusts were collected together and studied.

2. In the case of the Bombay Locust, it may be stated that most of the forms were met with in the areas of Rajputana, Sind and Western India States, and always either in autumn, winter or spring months, but never during the monsoon period. Though it is evident that the forms met with are migrants, the data available at present are not such as to lead to any definite conclusions.

3. In regard to the Migratory Locust, the data for 1931 to 1935 are rather fragmentary, but serve to indicate though vaguely that the *Locusta* population is most in evidence in the desert areas during the monsoon months, during which period breeding also takes place. In the Baluchistan areas *Locusta* is observable mostly in spring, when it breeds there generally. In case there should be summer rainfall, summer breeding also may occur.

4. The data for 1936-38 are very much fuller, and detailed observations are available. During May-June, 1937, there was intensive multiplication in the Kachhi, Bolan, and Kohlu areas of Baluchistan, but by August, the high population noticed in June had disappeared. In July, rather dense concentrations of *Locusta* adults were seen in east Bikaner and there was heavy multiplication in July-August there. Owing to the development of a drought in August, the population gradually disappeared during September. In September-October, there was a severe hopper infestation in millets in the Sirohi-Mehsana-Idar areas, which is presumed to have developed from locusts derived from the Bikaner areas. In November-December, large concentrations of adults were found in *jowari* fields in Kathiawar, Sind and Lasbela ; and in March 1938, none were to be found in Sind and Lasbela, though a fair number was seen in wheat fields in the Bolan Valley. From the above it is obvious that there has been a great shifting of population from one area to another during 1937, leading to considerable infestation.

5. Though no direct evidence in regard to migration has been available, there is much circumstantial evidence to show that the *solitaries* of *Locusta* are capable of long distance migration, like those of the Desert Locust. The sequence of events recorded in 1937, *viz.* the successive broods noted in May-June, July-August and September-November, and the concentrations of adults noted in *jowari* in November-December, and in wheat in March 1938, is a fairly clear indication of the migratory capacities of *Locusta*.

6. *Locusta* differs from *Schistocerca* in its ecological preferences, as it definitely favours a fairly moist environment, and has a special predilection for food-plants of the grass family. These preferences are of special significance, as they lead to the formation of its concentrations on irrigated crops like wheat and millets in hill valleys, and to the development of outbreak centres in such situations as a result of crowded breeding.

7. A study of the phase characters of the populations collected during different parts of the year 1937 has shown that the insect had undergone a certain degree of morphological change as a result of crowded breeding. In general the proportion of forms with '*intermediate*' E/F ratios as compared with the *solitaria* ones showed a definite rise and in one case (at Sirohi), definite '*gregaria*' ratios were found to have been developed.

8. The natural enemies observed consisted of the usual bird predators, certain bats and two kinds of Acarid mites.

9. Although the *solitaria* form of *Locusta migratoria* is common throughout India, there are no definite records of the development of swarms of *Locusta* in India, but a case recorded by Cotes in the *Indian Museum Notes* Vol. **2** (1891—1893) under the title " Madras Locust Invasion of 1878 " may possibly have been an instance of the swarming of this species.

10. The development of an infestation of locusts of the type of *Locusta* or *Schistocerca* is dependent on the existence of a combination of suitably situated summer and winter breeding grounds between which the seasonal winds can naturally bring about a regular transference of locust populations

at the end of the breeding period, rendering it possible for the locust to have both a summer and a winter or spring generation during the year. A serious infestation can, however, develop only in case the locust is able to greatly multiply in numbers as a result of two or more consecutive broods developing with the occurrence of exceptionally high rainfall in winter as well as in summer. It is, therefore, considered that the swarming of *Locusta* would, under the conditions of north-west India, be a rather exceptional event.

Acknowledgments

The authors take this opportunity of thanking the various authorities in Sind, Punjab and Baluchistan and the Western India States Agency, and the State Authorities in Kalat, Lasbela, Bikaner, Jodhpur, Jaisalmer, Jaipur, Sirohi, Idar, Palanpur, Baroda, the Kathiawar States, and Cutch for the help received in the course of the investigation. The senior author wishes specially to acknowledge the help received from Dr. V. N. Likhite in investigating the infestation in the Mehsana District. He is much indebted to Dr. H. S. Pruthi, Imperial Entomologist, New Delhi, for permission to examine the locust specimens in his collections. The authors wish to express their thanks to Sir Guy Marshall, Director, Imperial Institute of Entomology, London, for the determination of specimens sent, and, last but not least, to Dr. B. P. Uvarov for much help and advice.

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Appendix

TABLE I

Finds of Solitaria *specimens of the Bombay Locust* (Patanga succincta) *in the desert areas of North-West India during 1933—1938*

Year	Season	Month	Baluchistan area	Sind-Rajputana-Kathiawar areas	No. of specimens found
1933	Spring	March	*Nil*	Radhanpur (4th March 1933)	One specimens
				Cutch (11th March 1937)	Four specimen
1934	Spring	May	*Nil*	Cutch (4th May 1934)	Two specimens
	Autumn	November	...	Bikaner (Sardarshahr) (4th November 1934)	One specimen
1935	Winter	January-February	*Nil*	Thar-Mallani area—	
				(Virawah, 27th January 1935)	Two specimens
				(Harrah, 30th January 1935)	One specimen
				(Dhorimana, 12th February 1935)	One specimen
	Summer	June	*Nil*	Jaisalmer (Nokh, 4th June 1935)	One specimen
1936	Autumn-Winter	November-December	*Nil*	Bikaner (Sonpalsar, 7th November 1936)	One specimen
				Jaisalmer—	
				(Girasar, 20th November 1936)	Two specimens
				(Nokh, 12th December 1936)	One specimen
1937	Winter	January	*Nil*	Jaisalmer— (Tarana, 18th January 1937)	One specimen
		February	*Nil*	(Nokh, February 1937)	Seven specimens
				(Lathi area, February 1937)	Five specimens
	Spring	March	*Nil*	Bahawalpore (Mithra, 9th March 1937)	One specimen
		April	...	Jaisalmer (Baru, 11th April 1937)	One specimen
	Autumn	October	*Nil*	Bikaner (Bambu, 27th October 1937)	One specimen
		November	...	Mallani (Barmer, 27th November 1937)	One specimen
	Winter	December	...	Bikaner (Sardarshahr, 10th December 1937)	One specimen
				Mallani area (Nand, 28th December 1937)	One specimen
				Kathiawar (Amreli, 26th December 1937)	One specimen
1938	Winter	January-February	*Nil*	Jaisalmer (Nokh, 14th January 1938)	One specimen
				Bikaner (Sardarshahr, February 1938)	Three specimens
				Thar area (Virawah, 8th February 1938)	One specimen
				Kathiawar—	
				(Junagarh, 12th February 1938)	One specimen
				(Viramgam, 2nd February 1938)	Two specimens
	Spring	March	Kachhi (Dadhar, 23rd March 1938) 1 specimen	Bikaner (Sardarshahr, 6th March 1938)	Two specimens

TABLE II

Available data regarding the occurrence of Locusta migratoria *for the years 1931—1938 in the Baluchistan and Sind-Rajputana Desert areas of North-West India*

Year	Season	Baluchistan Region				Sind-Rajputana Region			
		Area	Month	Particulars	Rainfall	Area	Month	Particulars	Rainfall
1931	Spring	Mekran	April	Adults (Pasni, Ormara) 4 specimens	Fairly good	No data available			
			May	Adults and a few hoppers (Panjgur) 2 specimens					
	Summer-Autumn	Harnai	July	Adults (Shahrig) 10 specimens	Fair	No data available			
		Kachhi-Sibi	August	Adults (Jhatpat) 1 specimen					
1932	Winter-Spring	Mekran	April	1 specimen (Pasni)	Failure	No data available			
		Upper Baluchistan	April	Adults (Quetta) 2 specimens	Winter rain below normal				
	Summer-Autumn	Mekran (Kolwa)	September	Adults and a few hoppers (Kolwa)	Good rain in July	Bahawalpore State	November	Adult (Khairpur) 1 specimen	Good falls
						Jaisalmer State	November	A few adults (Sodakhor)	
1933	Winter-Spring	Mekran	April	Adults (Pasni) 2 specimens	Good rains	West Sind	February	Adults (Akil) 2 specimens	Fair
							May	Adults (Akil) 4 specimens	
						Bahawalpore State	June	Adults (Fort Abbas) 1 specimen	

TABLE II—*contd.*

Year	Season	Baluchistan Region				Sind-Rajputana Region			
		Area	Month	Particulars	Rainfall	Area	Month	Particulars	Rainfall
1933	Summer .	Lasbela .	July-Aug.	Adults 3 specimens	Good rain	West Sind .	July . .	Adults (Shah Hasan) 3 specimens	Heavy rainfall
		Mekran .	July .	Adults (Panjgur area) 8 specimens		Bikaner . .	July . .	Adults (Nohar) 4 specimens	
			August-September	Adults (Kolwa area) 5 specimens		Bahawalpur-Bhatinda	August-September	Adults specimens	
		Kachhi .	August .	Adults (Chattr) 1 specimen					
	Autumn .	Mekran .	September-October	Adults (Kech, Kalmalt) 5 specimens	...	Sind (Khairpur)	October .	Adults (Thari) 3 specimens	Good rain in September-October in desert
			November	Adults (Kech-Panjgur area) 6 specimens		Derajat . .	October-November	Adults (Mithankot Parao) 4 specimens	
			December	Adults (Buleda) 1 specimen		Sind (Thar area)	October . .	Adults (Chachro) 4 specimens	
						Jaisalmer .	October-November-December	Adults (Baru, etc.) 8 specimens	
						Bahawalpur	December .	Adults (Fort Abbas, Ft. Marot) 10 specimens	
						United Provinces (Muttra Distt.)	November .	Adults 1 specimen	
1934	Winter-Spring	Mekran-Baluchistan	May .	One adult (Panjgur) No other record of specimens anywhere in Mekran and Upper Baluchistan	Complete failure of Winter rains	West Sind .	April . .	Adults, a few (Mirzapur) 1 specimen	
						Cutch State .	May . .	Adults (Mandvi) 2 specimens	
						Bikaner . .	May . .	Adults (Suratgarh etc.) 6 specimens	
						Bahawalpur	May . .	Adults (Shahwala) 1 specimen	

	Summer-Autumn	Mekran .	July .	One specimen was collected at Pasni	No rain in Mekran	Sind (Thar area)	June-July .	Numerous adults (Chachro, Rahimki-Bazaar) 13 specimens	Monsoon began early in second half of June Rains light in August-September
		Lasbela .	November	One adult at Hingol	Light fall in Lasbela		August . .	Hoppers (Chachro) Adults (Diplo, etc.)	
						Punjab (Ludhiana)	July . .	Adults and hoppers reported from Ludhiana-Patiala area	
						Jaipur State .	September .	Adults (Sikar) 1 specimen	
						Sind (Thar) .	September .	Adults (Saro) 1 specimen	
						Bikaner area .	October . .	A few adults	
1935	Spring .	Mekran .	May .	One specimen collected at Mand	Good rainfall in winter	Bikaner . .	March . .	One specimen at Sardarshahr	...
	Summer .	Lasbela .	June .	One adult at Liari .	No rain in summer	Jaisalmer .	July . .	One specimen at Nokh	Fair
1936	Winter-Spring	...	No record	.....	Fair	.....		No record	
	Summer-Autumn	Kachhi .	July .	Fairly large numbers of adults were found in *jowari* cultivation in the Bhag-Gandhawa areas	No rain in Mekran	Bikaner area .	June . .	An adult (Udasar) .	Monsoon rather light
						West Sind .	July . .	Adults found in fair numbers at Johi in *jowari* fields	
		Lasbela .	November	One specimen collected at Goth Sherkhan	Light rainfall in Lasbela	Bikaner area .	August, September and October	Fair numbers met with during surveys along with the Desert Locust	Very heavy rainfall in East Jaisalmer and Bikaner areas in August 1936
						Jaisalmer areas	October, November and December	Found in good numbers with *Schistocerca* especially in November	
						Thar-Mallani area	August, September, October-November	Found along with *Schistocerca* in fair numbers ; none were noticeable in this area	

TABLE II—*contd.*

Year.	Season	Baluchistan Region				Sind-Rajputana Region			
		Area	Month	Particulars	Rainfall	Area	Month	Particulars	Rainfall
1937	Winter-Spring	Kachhi .	May-June	Adults and hoppers in fairly good numbers	Good spring rainfall in Upper Baluchistan	Thar-Mallani Desert areas	January to June	None found . .	Good falls in February
		Bolan Valley	June .	Very large numbers of hoppers and adults at Kirta		Bikaner-Jaisalmer, Bikaner Desert areas	January to May	Three specimens, observed in east Jaisalmer area, one in February, one in April and one in May	
		Mekran (Kolwa)	May .	Adults found in cultivation					
		Sibi Distt. (Kohlu)	May-June	Hoppers and adults reported		West Sind .	June . .	One adult at Johi	
		Chagai .	June .	A few adults found .		...	...	.....	...
		Lasbela .	June .	A few adults found .		...	...	.....	...
	Summer-Autumn	Kachhi and Bolan	August .	A small number of adults noted	...	Jaisalmer-west Bikaner	July	Present in fair numbers at the beginning of July, but few seen after the 25th	Light falls in July ; *nil* in August Light in September
		Harnai Valley	August .	Hoppers met with in fair numbers, as also some adults		...	August . .	Small numbers noted at the end of August	
						East and Central Bikaner area	July-August .	Enormous numbers of adults were found in certain parts, *e.g.*, Sadulpur ; there was also extensive breeding ; by end of August, large numbers of adults were noted in fields of *bajri*	Heavy rains in July ; none in August ; fair in September
							September .	Only small numbers of adults noticeable	
						Thar-Mallani area	July-August .	Both adults and hoppers found in fair numbers throughout the area	Fair rain in July ; *nil* in August ; good in September

						Sirohi; Gujarat (Mehsana) Kathiawar	September . October-November	Enormous numbers of hopper were found doing damage to *jowari* crops in Mehsana, Idar and Sirohi areas in October; by end of November, most of the adults had disappeared	Good rain in July, *nil* in August, heavy in September
	Winter	Lasbela-Karachi	November-December	Great numbers of adult locusts were noticed among *jowari* crops in many places	...	Kathiawar .	November-December	Small groups of adult locusts were found in a few localities in Kathiawar	...
1938	Winter-Spring	Lasbela .	February-March	Small numbers of hoppers were noticed in a few places	...	Cutch area .	January . .	Small numbers of adults in parts of the State	
	Spring .	Kachhi-Bolan-Harnai	March .	Small numbers of adults were found among wheat cultivation in several localities (egg-laying probable)	No spring rains in February and March	...	...	.....	...

TABLE III

Rainfall for the year 1936-37 (in inches)

Month	Panjgur	Turbat	Bela	Ambagh	Karachi	Gandhawa	Bhag	Harnai	Chachro	Barmer	Nokh	Sardar Shahr	Sadulpur	Churu	Mandar	Bikaner	Mehsana
1936																	
December .	0·26	1·05	3·33	2·13	0·10	1·48	0·85	6·39	*Nil*	*Nil*	*Nil*	0·02	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
1937																	
January . .	0·54	0·50	*Nil*	*Nil*	*Nil*	0·10	0·20	0·56	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
February .	1·60	1·20	1·46	0·92	1·00	1·34	1·00	1·38	0·29	0·61	2·22	1·46	1·53	0·65	*Nil*	2·10	0·15
March . .	0·18	0·22	0·70	*Nil*	*Nil*	0·90	0·21	1·29	*Nil*	*Nil*	0·03	0·18	0·15	*Nil*	*Nil*	*Nil*	*Nil*
April . .	0·29	0·34	*Nil*	*Nil*	*Nil*	0·96	0·26	0·87	*Nil*	*Nil*	0·02	0·04	0·04	0·21	*Nil*	*Nil*	*Nil*
May . .	*Nil*	*Nil*	*Nil.*	*Nil*	*Nil*	*Nil*	*Nil*	0·05	*Nil*	*Nil*	*Nil*	0·09	*Nil*	0·28	*Nil*	*Nil*	*Nil*
June . .	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	0·94	0·17	0·20	0·03	0·18	1·42	1·21	5·68	*Nil*	13·66
July . .	0·20	0·03	4·07	3·09	8·20	3·15	1·43	6·36	5·86	7·54	2·77	4·00	8·26	7·59	10·99	8·80	17·67
August . .	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	0·87	*Nil*	*Nil*	*Nil*	0·29	1·07	0·13	*Nil*	*Nil*	0·33
September .	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	2·13	2·63	1·36	1·91	2·01	2·54	2·24	10·13	0·90	15·70
October . .	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	0·63	*Nil*	*Nil*	*Nil*	0·02	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
November .	*Nil*	*Nil*	0·03	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
Total .	3·07	3·07	9·59	6·14	9·30	7·03	3·95	21·47	8·95	9·71	6·98	8·29	15·01	12·31	26·80	11·80	47·51

TABLE IV

Results of a biometrical analysis of Locusta *populations in North-West India in 1937*

Serial No.	Months	Area	Total No. examined	'*Solitaria*' ratios 1·90 and below	'*Intermediate*' ratios between 1·91 and 2·00	'*Gregaria*' ratios 2·01 and above
1	May-June . .	Sibi-Bolan-Kachhi areas	35	30	5 (14 per cent)	*Nil*
2	July . . .	Nokh-Jaisalmer-Bikaner areas	43	29	14 (32 per cent)	*Nil*
3	July-August . .	Thar-Mallani area .	21	19	2 (9 per cent)	*Nil*
4	September-October .	Bikaner-Jaipur-Jodhpur areas	37	35	2 (6 per cent)	*Nil*
5	October . . .	Sirohi area . .	26	6 (23 per cent)	8 (31 per cent)	12 (46 per cent)
6	November . .	Thar-Mallani . .	8	6	2 (25 per cent)	*Nil*
7	November . .	Mehsana (Baroda District)	28	7	21 (75 per cent)	*Nil*
8	November-December .	Lasbela area . .	65	50	15 (23 per cent)	*Nil*

ECONOMICS OF FIELD-SCALE SPRAYING AGAINST THE WHITE-FLY OF COTTON (*BEMISIA GOSSYPIPERDA* M. & L.)

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I. INTRODUCTORY

The White-fly, *Benisia gossypiperda* M. & L., is one of the major pests of cottons in the Punjab. It sucks sap from the leaves and drains the plant food. The honey-dew, which it secretes falls on the leaves and serves as a medium for the growth of black moulds, which interfere in photosynthesis. An attack of this pest is, therefore, capable of causing very heavy reduction in yield.

Small scale* experiments, conducted from 1929 to 1932, amply proved the value of spraying for the control of White-fly. In order to estimate the efficacy and economics of this method of control, on field scale, the Indian Central Cotton Committee sanctioned a sum of Rs. 11,250 for spraying trials during 1933. Extensive spraying operations were conducted during July and August, at the British Cotton Growing Association's Farm at Khanewal, and at the Military Farm, Okara. During 1934, the Committee sanctioned another grant of Rs. 6,590, which included a sum of Rs. 5,354 being the amount of savings from the previous year, for further spraying trials. The Committee desired that these trials be extended to four centres, namely Khanewal, Okara, Sargodha and Lyallpur, and that both the *desi* and American cottons be treated and that insecticides other than Rosin compound be tested. It is regretted that the operations could not be carried out at Lyallpur for want of spraying machines.

As far as we know, it is the first time that under Indian conditions, spraying operations have been conducted on so large a scale, in the field. The data presented, therefore, will be useful not only to cotton growers, but also to farmers in general.

The British Cotton Growing Association, Ltd., Khanewal, very kindly placed, at our disposal, their spraying machine (Sapom) free of charge. The Department of Agriculture lent the services of their mechanic and permitted the use of their Orchard Power sprayer and other equipment. One Hardie and one Sapom sprayer were purchased from the grant sanctioned by the Indian Central Cotton Committee during 1933. The generosity of the Committee is gratefully acknowledged.

The statistical analysis of the data was carried out by Mr. B. R. Sehgal, Statistical Assistant, Cotton Research Laboratory, Lyallpur. His help is gratefully acknowledged.

II. Experimental technique

The efficacy of spraying may be determined by comparing the yield from the sprayed fields with that from the unsprayed plots, and also by the actual determination of the percentage of insect mortality. The first method appeals to the grower as it enables him to determine his net gain. This method, however, has serious limitations. The yield of a crop depends upon a number of factors, such as climate, soil fertility, crop rotations, dates of sowing, frequency and quantity of rainfall and irrigation facilities and upon attacks of pests and diseases other than the one under investigation. It is impossible to expect any one of the above factors to be uniform, when one is dealing with large areas at different places. If one could vary the 'White-fly' factor alone, it would be possible to arrive at some definite conclusion, but often unexpected factors come in and vitiate the results of such experiments. For instance, during 1933, some of the sprayed fields

* The average size of plots in these experiments varied from 1/12th to 1/20th of an acre.

at Khanewal were so badly attacked by *Sylepta derrogata*, that the increase in yield as the result of spraying could not be definitely determined. Nevertheless, the data of yields from sprayed and unsprayed fields for the years 1933 and 1934 have been collected and given in Tables IV, V and VI.

The second method gives reliable data of insect mortality, but does not give any idea of the economic advantages of the operation. We have mostly depended upon this method to determine the efficacy of spraying. Mortality among the adults was estimated by counting the dead and living insects soon after spraying. For nymphs and pupae, samples of sprayed leaves were examined under a magnifying lens twenty-four hours after the operation. The natural insect mortality was determined by examination of similar samples of leaves from the neighbouring unsprayed plots.

III. Insecticides used

During 1933, 1280 acres of cotton crop at Khanewal and 521 acres at Okara, and in 1934, 1585 acres at Khanewal, 656 acres at Okara and 45 acres at Sargodha, were sprayed with rosin compound. The other insecticides used are listed below :—

Table I

Insecticides tried during 1933 and 1934

Insecticide	Formula adopted		Dilution tried
Rosin compound	(*a*) Rosin Grade III	5 lbs.	1 : 6
	Washing soda	1 lb.	
	Water	5 gls.	
	(*b*) Rosin Grade III	5½ lbs.	1 : 6
	Washing soda	1 lb.	
	Water	5½ gls.	
	(Used at Khanewal in 1934).		
Rosin soap	Rosin soap	2 lbs.	1 : 8
	Water	1 gl.	
Fish oil soap	Fish oil soap	2 lbs.	1 : 8
	Water	1 gl.	
Tobacco decoction	Tobacco	1 lb.	1 : 6
	Washing soap	½ lb.	
	Water	2 gls.	
Kerosene oil emulsion	Kerosene oil	½ gl.	1 : 9
	Washing soap	2 lbs.	
	Water	2 gls.	
Lime sulphur	Sulphur	5 lbs.	1 : 8
	Slaked lime	2 lbs.	
	Water	4 gls.	

Preliminary dusting trials were also carried out at Khanewal with calcium cyanide and nicotine sulphate during 1933, on an area of ten acres. In 1934, a number of proprietory dusts were tried at Khanewal on an area of twenty-three acres. The heavy cost, coupled with low mortality obtained from these dusts, did not encourage their further use.

IV. Spraying equipment employed

A cart sprayer can only be used when the crop is young and has been sown in lines, but when it has grown tall and is not sown in lines, it is practically impossible to take a machine through the field. To meet these two conditions, we tried two distinct types of spraying machines. The cart sprayers were represented by Hardie and Sapom sprayers, while the Orchard Power sprayer represented the second type. The following is a brief description of various machines and spraying equipment employed.

Cart sprayers

1. Sapom sprayer (cost Rs. 900 to Rs. 1,200).—In this machine the insecticide is forced out of five to seven nozzles, by a pair of pumps which work by means of a gear connected with the wheels of the cart. The force of the spray depends directly upon the speed with which the cart is moving. As the slow motion of the bullocks was not enough to produce the pressure required for a proper spray, the number of nozzles (at Khanewal) was reduced to 5 and they were worked by hand. Thus five boys were employed to work with 5 nozzles, each spraying one row of cotton. At Okara, four boys were employed to work with seven nozzles. Three boys sprayed with two nozzles each, while the fourth worked with a single nozzle. Spraying with two nozzles to a row gave better kill than with one nozzle to a row, although comparatively larger quantity of insecticide was used. The machine is useful, when the crop is not very bushy and trained labour is available.

2. Hardie Power sprayer (cost Rs. 1,100).—This machine consists of a 2 H. P. engine which pumps the insecticide through twelve nozzles, which spray four rows of cotton at a time. The sprayer works exceedingly well, as the nozzles are adjustable and automatic spraying very satisfactory. With a pressure of 75-130 lbs., it was possible to produce a very thorough cloud and spray almost all the leaves of a plant.

3. Orchard Power sprayer (cost Rs. 6,766).—This machine consists of a 36 H. P. engine and is designed to spray tall trees. We tried to adopt it for spraying a crop, when it had grown so tall and thick that a cart could not be taken in and it had to be sprayed from an engine stationed outside the field. The spraying outfit consisted of (*a*) delivery hose about 40 feet long (1 in. diameter), (*b*) a number of light steel pipes each 15 feet long (1 in. or $\frac{3}{4}$ in. diameter), (*c*) flexible armoured rubber joints with gunmetal screw couplings, which allowed the piping being laid on the roughest ground, (*d*) three-way cocks inserted at desired intervals, to the outlets of which were attached, (*e*) flexible

armoured hoses each 70 feet long and (*f*) specially designed horizontal spraying rods, with six nozzles. In Fig. 1, the distribution of pipes and hoses as laid in a cotton field and the rows of cotton sprayed by each rod are shown. One man, worked with one rod and sprayed three complete rows at a time. The rods were kept lifted up by means of a specially designed harness. To avoid any damage to the crop, by dragging the hoses, one additional man was employed at each hose to keep it lifted up.

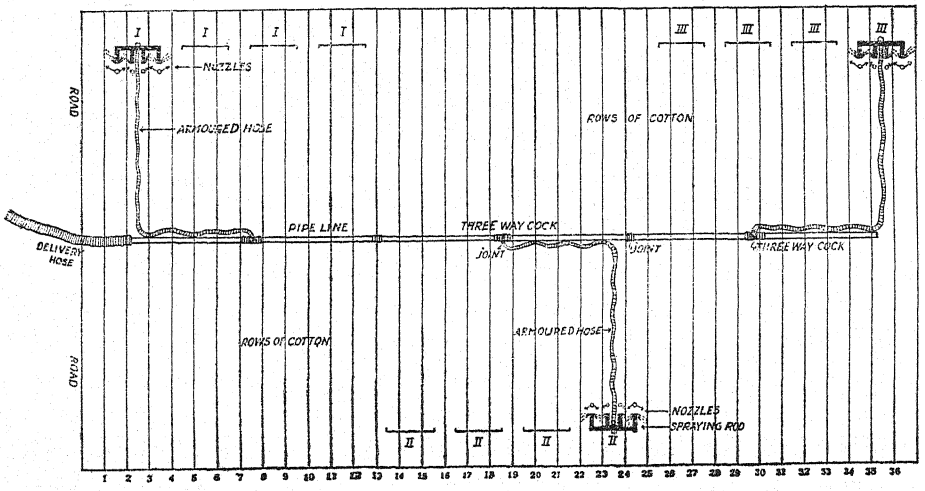

FIG. 1. Distribution of spraying outfit in a cotton field with orchard power sprays

Although spraying with this machine was very satisfactory, thorough and effective, yet its working was very cumbersome. Because of its heavy weight, it was difficult to move the machine from place to place, particularly when the tank was full and the field soft or wet. Further, the laying out of the pipes and their shifting was slow and troublesome. Leakage generally occurred at various joints and considerable time was lost in tightening these. Because of the heavy petrol consumption, and the necessity of employing two mechanics, spraying with this machine proved very expensive.

V. EFFICACY OF MACHINERY

The efficiency of various machines used in these trials was determined by studying the following points :—

1. Daily output of work.—The daily output of a machine mainly depends on its satisfactory working, speed of bullocks, efficiency of labour employed and the stage of the growth of the crop. At Khanewal (1933 and 1934), two pairs of bullocks were employed for each machine, on a contract to spray 15 acres a day. This arrangement proved satisfactory, as the deficiency on any day was made good subsequently. At Okara (1933 and 1934) and Sargodha (1934) bullock labour was engaged on daily basis.

During 1933, at Khanewal, each machine (Hardie and Sapom) sprayed on an average 15 acres a day. At Okara, however, because of the comparatively thicker crop, the average daily output with the Orchard Power sprayer was only 9·5 acres and with the Sapom 9·9 acres.

During 1934, an extra pair of bullocks was employed at Khanewal, during July, for a period of seven days and this raised the daily output of Hardie and Sapom to 16 acres. At Okara, the average area covered by the Orchard Power sprayer each day was 10·5 acres. This increase was on account of new hoses and pipe connections, which reduced leakage. The average daily output of Sapom was 19 acres.

2. Time factor.—The efficiency of a machine can be further judged by the time lost during working hours, in such operations as filling the tank, cleaning nozzles, and shifting the sprayer from field to field. During 1934, the loss of time with individual machines was as follows :—

The average time required for spraying one acre of cotton crop at Khanewal was twenty-four minutes with Sapom and twenty-six minutes with Hardie. Of this period, nineteen minutes were spent in actual spraying and the rest in filling the tanks, etc. It was calculated that the total time lost each day, in charging the tanks, shifting, cleaning, etc., was 36·4 per cent in the case of Hardie and 33·1 per cent in the case of Sapom.

At Okara, the actual time taken in spraying one acre with Sapom was thirteen minutes and with the Orchard Power sprayer 12·4 minutes. It was estimated from these figures, that 54·2 per cent of the time was lost in the case of Sapom and 75·4 per cent in the case of Orchard Power sprayer, in making preparation for spraying. The increased loss of time at this place in the case of Sapom, was due to the fact that often water had to be carried from a distance and also because the labour engaged on this machine was used in lifting the pipe line, while shifting the Orchard Power sprayer.

3. Mortality of the pest.—In young cotton crop, all the machines proved equally effective in killing the pest, but with the growth of the crop, only the power sprayers yielded satisfactory results. The Orchard Power sprayer was found to be most efficacious because of its good, steady pressure and controlled spraying of the bushes. With Rosin compound, 95·7 per cent of the adults and 93·9 per cent of the nymphs and pupae were killed in 1933, and 91·9 per cent of the adults and 84 per cent of the nymphs and pupae were killed in 1934. With Hardie sprayer, the maximum mortality was 92·8 per cent among adults and 87·4 per cent among nymphs and pupae during 1933, and 81·7 per cent among adults and 75·6 per cent among nymphs during 1934. With Sapom sprayer the mortality was comparatively low (Table II).

TABLE II

Percentage mortality among adults and nymphs of B. gossypiperda *M. & L., with different insecticides at Khanewal and Okara during 1933 and 1934*

Locality	Insecticides tried	Dates—Fortnight ending	Hardie		Sapom		Orchard Power Sprayer		Unsprayed	Remarks
			Nymphs and pupae	Adults	Nymphs and pupae	Adults	Nymphs and pupae	Adults	Nymphs and pupae	
Khanewal	Rosin compound	15th July	85·9	92·8	67·9	80·7	...	...	3·5	1933
		31st July	87·4	93·7	76·0	80·8	...	...	3·6	
		15th August	83·9	88·7	74·4	74·6	...	...	7·1	
		31st August	80·6	86·3	64·1	63·6	...	...	11·4	
		Average	84·4	90·3	70·6	74·9	...	...	6·4	
Okara	Do.	15th July	...	...	73·4	75·1	78·5	78·6	Ranged between 3·8 to 11·8	
		31st July	...	...	78·4	80·2	84·7	85·7		
		15th August	...	...	84·0	90·8	93·9	95·7		
		31st August	...	...	78·7	83·1	85·9	90·4		
		Average	...	...	78·6	82·3	85·7	87·6	7·2	
Khanewal	Do.	15th July	74·5	81·7	69·8	76·7	...	...	7·2	1934
		31st July	75·6	78·1	69·7	74·1	...	...	7·1	
		15th August	70·9	73·2	59·9	73·6	...	...	3·2	
		31st August	67·6	76·2	52·9	55·3	...	...	7·6	
		Average	72·1	77·3	63·0	69·9	...	...	6·2	
	Fish-oil-soap	15th August	70·6	71·2	51·5	...	...	...	...	
	Tobacco decoction	Do.	59·1	75·2	...	...	...	...	...	
	Kerosene oil emulsion	Do.	64·2	71·0	49·2	63·2	...	...	...	
Okara	Rosin compound	15th July	...	...	71·7	73·8	80·5	85·0	...	
		31st July	...	...	79·8	82·2	84·0	90·6	2·5	
		15th August	...	...	...	...	85·0	88·5	6·1	
		31st August	...	...	...	...	82·6	91·1	7·9	
		Average	...	...	75·7	78·0	83·0	88·8	5·1	
	Rosin soap	15th August	...	...	...	...	83·5	89·8	...	
	Tobacco decoction	Do.	...	...	...	...	71·0	63·8	...	
	Lime-sulphur wash	Do.	...	...	...	...	62·3	45·1	...	

4. Consumption of insecticide.—The consumption of insecticide per acre varied with each machine and, naturally, it also varied with the condition of the crop. During 1933, spraying operations were conducted from 11th July to 31st August, both at Khanewal and at Okara. The Hardie and Sapom sprayers at Khanewal used on an average, 58·2 and 43·3 gallons of insecticide per acre, respectively. The Sapom at Okara consumed 57·1 gallons per acre, while the Orchard Power sprayer consumed 68·4 gallons per acre. The crop at Okara was relatively bushy and more severely infested.

During 1934 the spraying operations were started a little earlier, *i.e.*, on 29th June at Okara and on 3rd July at Khanewal. The crop at the time was very young. The insecticide consumed by Hardie and Sapom sprayers at Khanewal was 51·8 and 43·4 gallons per acre, respectively. At Okara, the Sapom sprayer was used till the 22nd July, after which it was shifted to Sargodha. The insecticide used per acre by this machine during this interval was only 31·5 gallons. This estimate does not represent the average of the season. The Orchard Power sprayer also showed lower consumption, *viz.*, 44·8 gallons per acre, as special care was taken to reduce leakage by using new pipe connections and hoses.

5. Labour charges, fuel and petrol expenses.—During 1933, the cost of manual and bullock labour at Khanewal was calculated at Re. 0-4-0 per acre with Hardie sprayer and Re. 0-6-8 with the Sapom sprayer. At Okara, the cost was Re. 0-7-1 per acre with Sapom and Re. 0-10-8 with the Orchard Power sprayer. These expenses were reduced to Re. 0-3-11, Re. 0-4-8, Re. 0-3-8 and Re. 0-10-3 respectively during 1934 (Table III).

The fuel expenses at Khanewal during 1933 were estimated at Re. 0-1-4 per acre for Hardie and Re. 0-1-2 for Sapom. During 1934, these expenses were reduced by 40·7 per cent and 39·7 per cent, respectively.

The petrol expenses per acre increased from Re. 0-2-0 with Hardie and Re. 0-6-5 with Orchard Power sprayer during 1933 to Re. 0-2-5 and, Re. 0-6-11 respectively, during 1934 (Table III). The increase in petrol consumption was probably due to the machines having seen longer service.

VI. Efficacy of insecticides

The efficacy of an insecticide is judged by the mortality of the pest and its cost. It also depends indirectly on the spraying machine used.

During 1933, almost the entire area was sprayed with rosin compound except an area of 72 acres at Okara, which was sprayed with rosin soap. Both the insecticides were found to be equally effective in killing the pest, but the cost of rosin soap was higher. Dusting with calcium cyanide in the ratio of one part of insecticide to seven parts of road dust and with nicotine sulphate in the ratio of one to three gave an average mortality of 36·2 per cent and 32·7 per cent, respectively, in nymphs and pupae.

During 1934, a number of other insecticides were also tried and their relative cost per acre worked out (Table III). Rosin compound gave maximum mortality with the power sprayers. Rosin soap also gave equally satisfactory results. Fish-oil soap, used at Okara during first fortnight of August, was found to be as effective as rosin compound. Tobacco decoction and kerosene oil emulsion were tried at Okara in the first fortnight of August. The mortality in nymphs and pupae with these was found to be very low. Lime-sulphur was also tried in the second fortnight of August, but did not give satisfactory results.

The cost of insecticide per acre with power sprayers, during 1933, was lowest for rosin compound (Table III). For rosin soap, it was 125 to 154 per cent higher with Hardie and Sapom sprayers, respectively. The cost of Fish-oil soap, Tobacco decoction and Kerosene oil emulsion, when applied with Hardie sprayer in 1934, was found to be 300 per cent, 74 per cent and 71 per cent higher than rosin compound, respectively. Rosin soap, lime-sulphur wash and tobacco decoction, when applied with Orchard Power sprayer, also cost 152 per cent, 66 per cent and 65·5 per cent more than rosin compound applied with the same machine (Table III).

Beside these, a sample of 'Evergreen' spray received from Kerr & Co., Bombay, was given a trial over 7·5 acres at Khanewal. The insecticide was used in the dilution of 1 : 300 and mortality on an average worked out at 73·0 per cent among nymphs and pupae and 85 per cent among adults. The cost of insecticide was Rs. 3-3-0 per acre.

Rosin compound proved to be the most satisfactory insecticide, both from point of view of insect mortality and cost. Its application against this pest can therefore be recommended with confidence.

VII. Total cost of spraying

In calculating the total cost of spraying per acre, all the items of expenditure, *viz.*, insecticide, labour, petrol, fuel, supervision charges and depreciation, etc., have been taken into consideration (Table III).

During 1933, cost of spraying per acre of cotton crop with rosin compound varied from Re. 1-5-10 with Sapom and Re. 1-9-10 with Hardie sprayer at Khanewal, to Re. 1-11-0 with Sapom and Rs. 3-5-0 with Orchard Power sprayer at Okara. With rosin soap, the cost increased to Rs. 2-5-0 and Rs. 4-5-1 per acre with Sapom and Orchard Power sprayer, respectively. During 1934, the average cost of spraying one acre of cotton crop with rosin compound at Khanewal was Re. 1-2-3 with Sapom and Re. 1-7-9 with Hardie, while at Okara, it was Re. 0-14-3 with Sapom and Rs. 2-9-9 with Orchard Power sprayer. It was thus reduced by 8·1 per cent and 16·4 per cent with Hardie and Sapom, respectively, at Khanewal and by 21·1 per cent with Orchard Power sprayer at Okara. It is possible to reduce the cost still further by paying greater attention to details, such as labour, preparation of insecticide and supervision, etc.

TABLE III

Cost of spraying with different insecticides using different types of machinery during 1933 and 1934.

Locality	Machine used	Insecticide tried	Total area sprayed (acres)	Average daily output of work (acres)	Total solution used per acre (gallons)	Cost of insecticide per acre	Cost of fuel used per acre	Cost of petrol per acre	Pay of mechanic per acre	Labour charges including bullocks per acre	Depreciation charges on machinery, etc., per acre	Miscellaneous charges per acre	Cost of spraying per acre excluding cost of insecticide	Total cost of spraying per acre including all charges
						1933								
						Rs. a. p.	Rs. a. p.	Rs. a. p.	Rs. a. p.	Rs. a. p.	Rs. a. p.	Rs. a. p.	Rs. a. p.	Rs. a. p.
Khanewal	Hardie	Rosin compound	645	15·0	58·2	0-11-11	0-1-4	0-2-0	0-2-0	0-4-0	0-4-1	0-0-6	0-13-11	1-9-10
	Sapom	Do.	635	14·8	48·3	0-10-0	0-1-2	...	0-0-4	0-6-8	0-3-5	0-0-3	0-11-8	1-5-10
Okara	Do.	Do.	227	9·9	57·4	0-11-8	0-1-1	...	0-0-7	0-7-1	0-6-5	0-0-3	0-15-5	1-11-1
	Do.	Rosin soap	51	9·9	57·4	1-6-0	0-0-5	...	0-0-7	0-7-1	0-6-7	0-0-3	0-14-9	2-5-0
	Orchard Power sprayer	Rosin compound	294	9·5	68·4	0-13-6	0-1-4	0-6-5	0-5-1	0-10-8	0-14-8	0-1-4	2-7-6	3-5-0
		Rosin soap	21	9·5	68·4	1-14-6	0-0-5	0-6-5	0-5-1	0-10-5	0-14-8	0-1-7	2-6-7	4-5-1
						1934								
Khanewal	Hardie	Rosin compound	807	16·1	51·8	0-11-9	0-0-10	0-2-5	0-0-10	0-3-11	0-3-8	0-0-4	0-12-0	1-7-9
		Fish oil soap	40	...	...	2-3-0	0-0-5	0-2-5	0-0-10	0-3-6	0-3-8	0-0-4	0-11-2	2-14-2
		Tobacco decoction	30	...	...	1-4-5	0-1-1	0-2-5	0-0-10	0-4-4	0-3-8	0-0-4	0-12-8	2-1-1
		Kerosene oil emulsion	18	...	...	1-4-1	0-0-6	0-2-5	0-0-10	0-4-0	0-3-8	0-0-4	0-11-10	1-15-10
	Sapom	Rosin compound	779	16·8	43·4	0-9-8	0-0-8	...	0-0-3	0-4-8	0-2-11	0-0-1	0-8-7	1-2-3
		Fish oil soap	70	...	...	1-12-0	0-0-5	...	0-0-4	0-4-11	0-2-11	0-0-1	0-8-8	2-4-8
		Kerosene oil emulsion	12	...	...	1-0-1	0-0-6	...	0-0-3	0-5-4	0-2-11	0-0-1	0-9-1	1-9-2
Okara	Do.	Rosin compound	342	19·0	32·0	0-6-5	0-0-8	...	0-0-3	0-3-8	0-3-2	0-0-1	0-7-10	0-14-3
	Orchard Power sprayer	Rosin compound	313	10·5	44·8	0-9-0	0-0-10	0-6-11	0-3-4	0-10-3	0-10-3	0-1-2	2-0-9	2-9-9
		Rosin soap	102	...	...	1-6-9	0-0-5	0-6-11	0-3-4	0-11-0	0-10-3	0-1-2	2-1-1	3-7-10
		Tobacco decoction	31	...	...	0-14-10	0-1-1	0-6-11	0-3-4	0-9-0	0-10-3	0-1-2	1-15-9	2-14-7
		Lime-sulphur wash	21	...	...	0-15-1	0-0-5	0-6-11	0-3-4	0-8-0	0-10-3	0-1-2	1-14-1	2-13-2

Of the three machines tried during these operations, Hardie Power sprayer was considered to be the most suitable machine for spraying cotton crop. The Sapom was ruled out on account of the low pest mortality obtained, while the Orchard Power sprayer was found to be both expensive as well as cumbersome.

VIII. Yield tests

From a grower's point of view, a control measure is worth adopting only if it brings increased yield sufficient to justify trouble and expenditure on the measure adopted. In order to judge the beneficial affects of these trials, the yield from sprayed and unsprayed fields at all the centres of operation, were collected and statistically compared (Tables IV, V and VI).

During 1933-34, at Khanewal (Table IV) the average increase in yield per acre by spraying American cottons was about one maund. At Okara, where both American and *desi* cottons were sprayed, the average increase in yield per acre in the former type was also one maund. In area under *desi* cotton, sprayed from 11th to 30th July, the maximum increase in yield per acre was 2·22 maunds, but in the area sprayed in the end of August negative results were obtained.

During 1934-35, at Khanewal (Table V) the average increase in yield per acre in American cotton was 1·72 maunds by single spraying and 2·07 maunds by double spraying. For *desi* cotton an average increase of 0·42 maund per acre was obtained. This low increase was probably due to the fact that about 25 per cent of the area was sprayed after 10th of August, an unsuitable time for spraying. The average increase in yield per acre, by spraying *desi* cotton during July was 1·56 maunds. American cotton sprayed in August gave an average increase of 2·03 maunds per acre. This was due to the fact that White-fly attack on these types was very severe in August and spraying was very effective.

At Okara, a nominal increase of 0·34 maund per acre was obtained by spraying American cotton and 2·0 maunds per acre by spraying *desi* cotton (Table VI). The low increase in the former type was on account of the early spraying (July) in Chak No. 4. The crop at this time was very young and the attack of the pest almost nil. Spraying the crop as early as that had no beneficial effect because the plants were subsequently exposed to White-fly attack almost to the same extent as if they had not been sprayed at all. Spraying after 15th July proved better and resulted in an increase of 0·71 maund per acre. There was an increase of 0·73 maund per acre in crop sprayed during August.

TABLE IV

Comparative yields of cotton from sprayed and unsprayed areas during 1933-34

Locality	Type of cotton	Chak No.	Sprayed		Unsprayed		Average increase in yield per acre in maunds	Statistical analysis			Statistical remark	Net return to the cultivator in Rs. due to increase in yield per acre	Remarks.
			Total area in acres	Average yield per acre in maunds	Total area in acres	Average yield per acre in maunds		No. of degrees of freedom 'n'	Fisher's 't'	Probability 'P'			
												Rs. a. p.	
Khanewal	American	75	274·3	13·20	...	...	...						
		81	148·0	11·85	...	...	...						
		82	292·9	12·10	102·7	11·82	+0·28						
		83	163·1	13·40	25·90	11·40	+2·00						
		85	120·7	11·73	38·0	11·05	+0·68						
		86	139·3	13·95	...	...	...						
		87	136·7	12·88	...	...	...						
		Average		12·73	...	11·42	+1·31	6	2·471	$0·05>P>0·02$	Significant	7-0-8	Calculated @ Rs. 5-6-0 per maund

Okara .	American	3	156·0	8·93	16·1	7·66	+1·27					
		4	146·4	9·33	80·6	8·34	+0·99					
		12	50·8	12·25	36·2	11·50	+0·75					
		Average		10·17	...	9·17	+1·00	2	6·687	0·05>P>0·02	Do. .	5-6-0 Ditto.
	Desi .	2	109·9	10·06	10·6	9·76	+0·30					
		4	80·1	11·46	110·8	9·24	+2·22	...	...	...	...	. Sprayed from 11th to 30th July
		12	56·1	9·90	57·2	10·05	—0·15	...	...	...	...	... Sprayed after 3rd week of August
		Average		10·47	...	9·68	+0·79	2	1·087	0·4>P>0·3	Not significant	3-5-9 Calculated @ Rs. 4-4-0 per maund

TABLE V

Comparative yields of cotton from sprayed and unsprayed areas at Khanewal during 1934-35

Type of cotton	Chak No.	Month and type of spraying	Period of spraying	Sprayed		Unsprayed		Average increase in yield per acre in maunds	Statistical analysis			Statistical remark	Net return to the cultivator in Rs. due to increase in yield per acre	Remarks
				Total area in acres	Average yield per acre in maunds	Total area in acres	Average yield per acre in maunds		No. of degrees of freedom '*n*'	Fisher's '*t*'	Probability '*P*'			
													Rs. a. p.	
American .	81	July (Single sprayed)	13th to 18th July	110·4	15·67	39·9	13·72	+1·95						
	82		19th July to 1st August.	161·2	12·75	63·5	11·42	+1·33						
	83		3rd to 10th July .	34·3	8·0	37·8	7·37	+0·63						
	85		11th to 12th July	23·1	7·95	42·2	6·27	+0·68						
			Average . .	...	11·10	...	9·70	+1·40	3	2·825	$0·1>P>0·05$	Not significant	9-8-7	Calculated @ Rs. 6-13 per maund
	75	August (Single sprayed)	11th to 19th Aug.	201·8	11·47	89·5	10·72	+0·75						Average increase for American cotton by single spraying was 1·72 mds. per acre
	85		3rd to 5th Aug. .	40·6	9·22	42·7	6·27	+2·95						
	86		20th to 27th Aug.	146·1	10·55	13·6	8·42	+2·13						
	87		25th to 26th Aug.	40·0	9·10	81·6	6·80	+2·30						
			Average . .	...	10·08	...	8·05	+2·03	3	3·727	$0·05>P>0·02$	Significant.	15-10-8	Calculated @ Rs. 6-13 per maund

	82	Double sprayed	19th July to 1st Aug. and 28th to 31st Aug.	99·7	12·55	63·5	11·42	+1·13						
	83		3rd to 10th July and 6th to 10th Aug.	114·8	8·53	37·8	7·37	+1·16						
	85		11th to 12th July and on 2nd Aug.	26·3	10·18	42·7	6·27	+3·91						
			Average . .	...	10·42	...	8·35	+2·07	2	1·308	0·4>P>0·3	Not significant	15-11-0	Ditto.
Desi .	81	July (Single sprayed)	13th to 18th July	51·7	16·87	14·8	14·32	+2·55						
	82		19th July to 1st Aug.	124·8	14·22	30·3	13·27	+0·95						
	83		3rd to 10th July .	64·3	7·45	21·3	6·40	+1·05						
			11th to 12th July	6·9	10·17	34·9	8·52	+1·65						
			Average . .	...	12·18	...	10·62	+1·56	3	4·272	0·05>P>0·02	Significant	8-6-10	Calculated @ Rs. 5-7 per maund
	75	August (Single sprayed)	11th to 19th Aug.	61·5	13·22	7·0	14·37	+1·05						
	85		3rd to 5th Aug. .	27·2	8·75	34·9	8·52	+0·23						
	86		20th to 27th Aug.	31·8	10·82	45·7	13·12	—2·30						
			Average . .	...	10·96	...	12·00	—1·04	2	1·425	0·3>P>0·2	Not significant	...	Average increase for *desi* per acre was 0·42 maund

TABLE VI

Comparative yields of cotton from sprayed and unsprayed areas at Okara and Sargodha during 1934-35

Locality and type of cotton	Chak No.	Month and type of spraying	Period of spraying	Sprayed		Unsprayed		Average increase in yield per acre in maunds	Statistical analysis			Statistical remarks	Net return to the cultivator in Rs. due to average increase per acre	Remarks
				Total area in acres	Average yield per acre in maunds	Average area in acres	Average yield per acre in maunds		Degrees of freedom 'n'	Fisher's 't'	Probability 'P'			
Okara—	4	July (Single sprayed)	28th June to 15th July.	175·8	8·00	16·0	8·44	+0·44					Rs. a. p.	
American .	12		16th to 31st July	101·5	8·12	17·0	7·41	+0·71						
			...	21·8	6·72	10·3	6·75	—0·03						
			Average . .	...	7·61	...	7·54	+0·07	2	0·226	$0·9>P>0·8$	Not significant	4-14-0	Calculated @ Rs. 6-13 per maund
	3	August	1st to 24th Aug. .	114·5	7·48	10·3	6·75	+0·73						
	3	(Double sprayed)	28th July to 23rd Aug. and 15th to 22nd Aug.	15·8	7·55	10·3	6·75	+0·85						
	4		28th June to 24th July and 25th to 28th Aug.	19·2	8·35	16·0	8·45	+0·10						
			Average . .	...	7·94	...	7·60	+0·34					4-5-1	Ditto. Replications too few

Desi .	4	July .	28th June to 15th July.	173·4	9·17	35·2	7·27	+1·90					
	12		16th to 31st July.	87·2	11·88	36·6	9·01	+2·87					
	3		...	12·2	10·35	86·4	8·84	+1·51					
			Average . .	...	10·47	...	8·37	+2·10	2	5·177	0·05>*P*>0·02	Significant	11-6 8	Calculated @ Rs. 5-7 per maund
Sargodha—American	...		28th July to 7th Aug.	27·3	11·05	10·0	9·18	+1·87						
Desi .			28th July to 7th Aug.	13·0	6·93	24·7	6·45	+0·48						
	Square No.													
American	34/1/½	...	...	0·431	13·27	0·431	12·87	+0·40						
	34/2/½	...	...	0·431	12·57	0·431	10·01	+2·56						
	A.B.8/2	...	...	1·250	11·01	1·250	8·42	+2·59						
	11E. ½	...	...	1·250	13·47	1·250	11·02	+2·45						
	34/8	...	...	0·550	19·40	0·500	15·60	+3·80						
			Average . .	...	13·94	...	11·58	+2·36	4	4·303	0·02>*P*>0·01	Significant	16-1-3	Calculated @ Rs. 6-13 per maund
Desi .	34/3/½	...	...	0·606	11·07	0·606	9·27	+1·80						
	A/3	...	...	1·231	12·85	1·231	9·45	+3·40						
			Average	...	11·96	...	9·36	+2·60	Replications too few				15-2-2	Calculated @ Rs. 5-7 per maund

Spraying the *desi* cotton during July proved quite effective, yielding an average increase of 2·10 maunds per acre.

At Sargodha, single spraying of American cotton gave an average increase of 2 maunds per acre, while for *desi* cotton, there was an increase of 0·48 maund only per acre. This was on account of the late spraying of this type (Table VI).

Further experiments were conducted at Sargodha, to test the efficacy of spraying in the following manner. In four squares each line under American cotton was divided lengthwise in two equal halves and in the fifth square it was divided across with equal number of cotton rows. Half of each line was sprayed and the corresponding half was left as control. Thus an area of 4·26 acres was sprayed in five different squares. In this way, most of the fluctuating factors, *viz.*, rotations, soil variations, irrigation and date of sowing, etc., were to a great extent eliminated. There was an average increase of 2·36 maunds per acre for American cotton and 2·60 maunds per acre for *desi* cotton.

It is clear from the above data that spraying the cotton crop with Rosin compound is beneficial against White-fly of cotton and results in increased yield. But the time of spraying is an important factor. The best time for spraying *desi* cotton is July, while spraying in August is definitely harmful. For American cotton, single spraying in August, before the crop has grown too bushy, is good, but double spraying, once in July and again in August, is better.

The net gain to the cultivator from the increase in yield per acre, calculated on the basis of prices prevailing in 1933 and 1934 for American and *desi* cottons, is given in the tables for yield data.

IX. Summary

Field-scale spraying trials were conducted against *B. gossypiperda* M. & L., a serious pest of cotton in the Punjab. A number of insecticides were tried.

Spraying the infested crop with rosin compound gave very satisfactory results. The cost of spraying with different types of machinery and insecticides has been worked out.

In all 1,873 acres of cotton crop were sprayed during 1933 and 2,460 acres during 1934. Machines of the type of cart sprayers proved better. Hardie Power sprayer with a 2 H. P. pump was found to be the most satisfactory.

Mortality of the pest was, on the whole, greatest with Orchard Power sprayer. The Hardie sprayer came next.

The cost of spraying with rosin compound varied from Re. 1-2-4 per acre with Sapom to Re. 1-7-8 with Hardie and Rs. 2-9-9 with the Orchard Power sprayer. It is possible to reduce the cost still further by paying more attention to details.

Spraying *desi* cotton in July and American cotton in August proved effective in increasing the yield. The net gain per acre to the cultivator justified expenditure on spraying.

NOTES ON THE LIFE-HISTORY AND HABITS OF *DACUS BREVISTYLUS* BEZZI (FAMILY TRYPETIDAE), A PEST OF *COCCINIA INDICA* FRUITS

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(With Plate VI)

INTRODUCTION

COCCINIA (*CEPHALANDRA*) *INDICA* known in Telugu as *dondakaya* and in Tamil as *kovaikkai* is one of the vegetable crops in the Northern Circars and was introduced into Coimbatore not long ago. This cucurbit creeper grows well in loamy red soils under irrigated conditions. The plant is generally raised as an annual but it can also be kept as a perennial crop for over three or four years. The first harvest comes in April. On an average there will be about fifteen pickings in a season, the maximum yield being obtained in April and May. The pickings are continued till September. In Coimbatore this fruit is subject to the attack of a Trypetid—*Dacus brevistylus*—and the present paper gives information on the life-history and habits of this pest under South Indian conditions.

HISTORY OF THE PEST

Dacus brevistylus belonging to sub-family Dacinae was originally described by Bezzi [1908]. This species is widely distributed in Africa and Europe. Bezzi [1914] for the first time recorded this species from India on melon from Sidhout (Cuddapah District). Silvestri [1914] gave a brief description of the adult flies. Senior White [1924] recorded a few more localities in India where the fly had been observed. The first record of the pest on *Coccinia* fruit is by Ramakrishna Ayyar [1935].

Description of the pest

The description of the fly by Silvestri [1914] is given below :—

Female (Fig. 9).—" Body more or less dark fulvous in colour ; face with two large black spots, and one brown spot on the peristome near the lower part of the eyes ; thorax with the scutum a little darker than the rest of the body, with a median presutural line and two sub-median post-sutural black spots, which may be more or less evident (very evident in the alcoholic specimens, almost invisible in those preserved dry) ; humeral calli, suture, pleurae opposite the suture, hypopleural spot and the scutellum yellowish white or ochraceous. Wings similar to those of the preceding species—*Dacus vertebratus* Bezzi. Scapulars in two pairs. Seventh segment 1·5 mm. ; ovipositor 1·56 mm. in length. Length of body 6-7 mm. "

Male (Fig. 8).—" Sides of the third abdominal segment provided with a posterior row of setae. Forceps of the copulatory organ with the outer side of the lower lamina longer than the inner, attenuated and arcuate. "

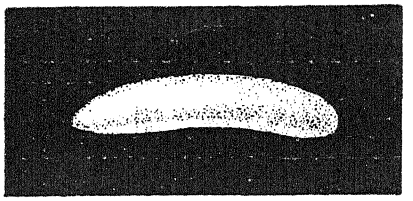
Habits of the pest

The time of the emergence of the adults both in the field and in the laboratory was noted to be from 8 to 10 A.M. From the observations made so far the pre-oviposition period seems to be at least four days.

The female is very active just prior to laying eggs. It goes about over the fruits a number of times and selects a convenient spot for egg laying which is very often near the stalk. This habit probably facilitates the dropping of the fruits at the time the maggots are full grown and ready for pupation which, under normal conditions, takes place in the soil. The female, after selecting the spot for oviposition, stretches out its ovipositor, with which it pricks the fruit and a liquid of dry white colour oozes out of the puncture. The female flies off for a while, comes back and licks up the fluid collected in the form of a globule. Again she thrusts her ovipositor in the same place and moves it up and down a number of times. In one puncture as many as eight eggs are laid. Three such punctures have been noticed in a single fruit containing a maximum of twenty-two eggs. When the maximum number of eggs exceeds ten or twelve, the maggots which hatch out and feed inside the fruit do not get enough food, with the result that the pupæ and adults resulting from these are under-sized.

The liquid oozing out through the puncture made on the fruit for oviposition dries up in the form of a crest, dark brown in colour. Two days after egg laying a small hollow is seen on the fruit at the place of oviposition, and the adjoining area begins to turn soft and brown. In about four or five days the whole fruit turns brown by which time the maggots have completely devoured its contents, leaving only the shrivelled-up skin and seeds. If the maggots are only a few in number a portion of the fruit still remains fresh but the fruit in either case drops to the ground. Within a day the maggots come out of the fruit and enter the soil for pupation.

1



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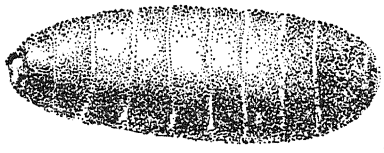

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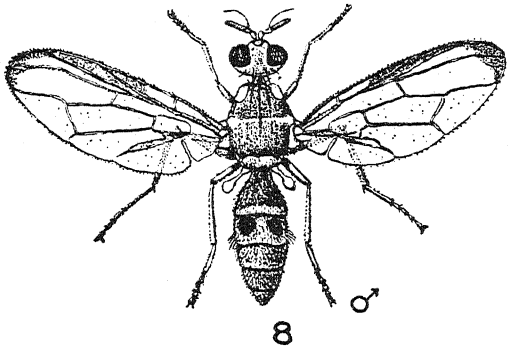

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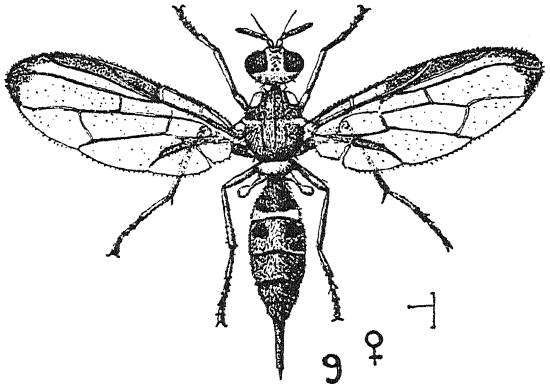
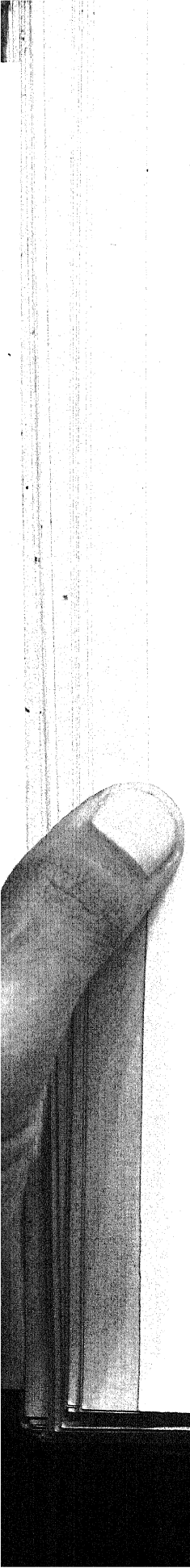


9 ♀

1. *Coccinia* fruit ; 2. Transverse section of fruit showing eggs ; 3. The egg (×7) ; 4. Just hatched maggot (×7) ; 5. Full grown maggot (×4) ; 6. Longitudinal section of fruit showing maggots ; 7. Puparium (×6) ; 8. Adult male (×3½) ; 9. Adult female (×3½)



LIFE-HISTORY

Egg (*Plate VI, figs. 2 & 3*).—Generally three to four eggs are laid in each puncture, the eggs being found adhering to the seed. The egg is about 2·5 mm. in length, shiny white, slightly curved, cylindrical in shape and narrow at one end. The incubation period varied in the laboratory from a little less than two days to four days between 27th September and 17th October 1935 (eleven observations).

Maggot.—The freshly hatched maggot (Plate VI, fig. 4) has a tapering head and black hook-like mouth-parts and is about the same size as the egg. When full grown (Plate VI, fig. 5) it is 8 mm. long and 1·4 mm. broad. According to Silvestri [1914] the mandibular hooks of the mouth-parts are provided each with a pre-apical tooth and the oral lobes, which are well developed, have each fifteen laminate ridges. There are several dorsal spines on the first three post-cephalic segments and a few on the fourth and the fifth. Each anterior spiracle is provided with fourteen to sixteen lobes. The maggots when full grown drop either with the fruit or by themselves and pupate in the soil. In the field, though the maggots drop in one place from a fruit, they disperse slightly before they enter the soil for pupation. The depth of the soil at which they pupate varies from half to two inches depending on the hardness or looseness of the soil. The larval period in the laboratory was four to six days in October 1935 (eight observations).

Puparium.—The puparium (Plate VI, fig. 7) is elliptical and brownish in colour and about $4\frac{1}{2}$ to $5\frac{1}{2}$ mm. long and 2 to $2\frac{1}{2}$ mm. broad. The pupal period was eight to ten days (nine observations).

Longevity of flies.—Longevity records of female and male flies kept in cages and fed with honey solution show that during October, November and December, the maximum longevity of a male was sixty days and that of a female forty-two days, the average for thirty flies of both sexes being twenty-eight days. The adult flies lived without food for three or four days only.

Total life-cycle.—The life-history data have been summarised in Table I. It will be seen that the total life-cycle of the fly ranges from fifteen to seventeen days. The temperature and humidity at which the life-history of the fly was studied are given in Table II. Under Coimbatore conditions there are about six broods in the year.

TABLE I

Life-history records of Dacus brevistylus

Egg laid on	Hatched on	Egg period (days)	Pupated on	Larval period in days	Adult emerged on	Pupal period (days)	Total life-cycle in days
9th Oct. 1935 .	11th Oct. 1935 .	2	15th Oct. 1935 .	4	24th Oct. 1935 .	9	15
10th Oct. 1935 .	12th Oct. 1935 .	2	15th Oct. 1935 .	4	24th Oct. 1935 .	9	15
11th Oct. 1935 .	13th Oct. 1935 .	2	17th Oct. 1935 .	5	27th Oct. 1935 .	10	17
12th Oct. 1935 .	14th Oct. 1935 .	2	18th Oct. 1935 .	5	28th Oct. 1935 .	10	17
14th Oct. 1935 .	16th Oct. 1935 .	2	21st Oct. 1935 .	6	29th Oct. 1935 .	8	16
17th Oct. 1935 2-20 P.M. .	19th Oct. 1935 10-15 A.M. .	One day and 2 hrs.	23rd Oct. 1935 .	5	1st Nov. 1935 .	9	16
18th Oct. 1935 .	20th Oct. 1935 .	2	24th Oct. 1935 .	5	2nd Nov. 1935 .	9	16

TABLE II

Temperature and humidity at which the life-history of Dacus brevistylus *was studied*

	September 1935	October 1935	November 1935	March 1936
Mean minimum temperature in shade (°F.) . . .	62·2	70·9	68·1	70·5
Mean maximum temperature in shade (°F.) . . .	89·3	87·8	86·1	94·1
Mean humidity at 8 hours (per cent)	69·1	78·9	74·2	73·4

OTHER FOOD PLANTS OF THE PEST

The fly has been noted to breed in captivity on snake-gourd, bitter-gourd, cucumber and melons. From field collections of these fruits also adults have been reared out.

OTHER FLIES REARED FROM *COCCINIA* FRUITS

In addition to *Dacus brevistylus* found in large numbers on this fruit, the cucurbit fly, *Dacus cucurbitae* Coq., has also been reared occasionally.

PARASITES

From fruits collected from the field for over a period of eight months, only half a dozen hymenopterous parasites of the species *Opius incisi* were collected. But it is not possible to say definitely that these are parasites of *Dacus brevistylus*, as from the infested fruit from which parasites were bred two specimens of *Dacus cucurbitae* also emerged.

LOCALITIES IN WHICH THE PEST HAS BEEN NOTED

The fly has been noted in the following places in the Madras Presidency :— Coimbatore, Kollegal, Mettuppalayam and Vellaikkinar in Coimbatore District ; Adoni and Hagari in Bellary District ; Taliparamba in Malabar ; Tripli-cane (Madras) ; and Sidhout (Cuddapah District).

REMEDIAL MEASURES

Trials of essential oils such as citronella to attract adult flies were not successful. In the field, it is easy to distinguish the attacked fruits from the unattacked ones by the characteristic straw-colored, dried-up liquid on the former. Such attacked fruits can be hand-picked and destroyed to reduce further infestation.

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A REVIEW OF THE LITERATURE ON THE NUTRITIVE VALUE OF PULSES

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THE pulses form a very important constituent of the dietary all over India. For various reasons, the people as a rule, are not in the habit of taking much proteins of animal origin and as such depend on vegetable proteins, the bulk of which comes from pulses. Until recently there has not been done much work on the investigation of the nutritive value of the different varieties of pulses that are grown in India. The *Health Bulletin* No. 23 [1937] embodies the composition of different pulses and shows their nutritive value on the basis of total protein, mineral and vitamin contents. " Shanghai Foods ", special report series No. 8 [1937] of the Chinese Medical Association also mentions the composition of some of them, as worked out by the Chinese workers. But it is erroneous to assess the nutritive value of pulses as a source of body-building material and also for making up the wear and tear of tissue in the process of living, on the basis of total protein in them. It is equally important to know the biological value, digestibility and the percentage of the essential amino-acid components of the beans. These problems have been studied by very few workers like, Niyogi *et al.* [1931, 1,2 ; 1932], Basu and his associates [1936 ; 1937] and Swaminathan [1937, 1,2] in India ; Adolph and Kiang [1920], Tso [1927], Kim [1928], Lo [1934], Chen [1930] and Adolph and Chiang [1935] in China ; Osborne and Mendel [1907, 1908], Sherman [1929] and Heller [1927] in America ; and Chick and Delf [1919], and Miller and Robins [1934] in England.

The quantitative analytical results of workers of different countries differ a good deal and even amongst Indian workers (Niyogi *et al.* at Bombay, Basu and co-workers at Dacca in Bengal and Swaminathan at Coonoor in South India), there is no uniformity of quantitative estimations. However, it is interesting to compare the results obtained in India by these workers. Table I shows a comparative study of the digestibility coefficient, biological value, and net protein value (side by side with the total protein content), at 10 per cent level of intake of eleven different pulses commonly used in India.

The most important protein fraction of the pulses is a globulin moity. Niyogi and co-workers [1931, 1, 2 and 1932] have found out the percentage composition of the different amino-acids of the globulin of the pulses expressed as per cent of protein. Table II shows the amino-acid constitution of common Indian pulses on a comparative basis, as analysed by Niyogi *et al.* Basu *et al.* [1937] found out the amino-acid constitution of the glutelin factor of two of the pulses, green gram (*Phaseolus radiatus*) and lentils (*Lens esculenta*) and that of the proteins of khesari (*Lathyrus sativa*) precipitated at different saturations of ammonium sulphate. These figures have been incorporated in Tables III and IV respectively.

If we consider the merit of different pulses as based on the results enumerated in the above tables, from different points of view, such as total protein content, biological value, digestibility, net protein value and percentage of the four important essential amino-acids (cystine, tyrosine, tryptophane and histidine), the different members of the group will range themselves in the order shown in Table V.

From the tabulated list (Table V) of different pulses it is rather difficult to say which is the best pulse as a source of protein available for tissue building. If, however, we allot two marks each for biological value and digestibility and one mark for each of the total protein content, net protein value, and percentages of cystine, tyrosine, tryptophane and histidine it is possible to obtain a rough indication as to which are the best types. Table VI shows the respective merits of different pulses as a result of allotment of marks on lines indicated above.

Bengal gram (*Cicer arietinum*) and black gram (*Phaseolus mungo*), both stand first in order of merit and green gram (*Phaseolus radiatus*), lentil (*lens esculenta*), cow pea (*Vigna catjang*) and soya bean (*Glycine hispida*) stand second in the list. This finding is quite in accordance with what Niyogi, Narayana and Desai [1931] observed regarding *Cicer arietinum* " that it is not only more digestible but its proteins are better assimilated than those of other pulses..... *Cicer arietinum* appears to be the best protein food because of its high net protein value." Heller [1927] also found the *mung* bean protein superior to that of many members of the bean family though it was deficient in cystine. Tso [1927] also studied the *mung* bean (*Phaseolus*

mungo L. ?) and while he found the proteins biologically complete, he stated that it was deficient in lime and sodium chloride although it was rich in vitamin B and had a moderate amount of vitamin A. So also Kim [1928] has shown that by germination the sprouted *mung* bean develops a high content of vitamin B and C. The finding of Heller [1927] that *mung* bean is deficient in calcium does not find confirmation in the hands of workers at Coonoor [*Health Bulletin*, No. 23] according to whom the mineral contents of the Bengal gram and green gram are almost the same, with this difference only that the carotene (vitamin A) content of the first is much higher than that of the second, whereas, vitamin B content of the latter is much higher than that of the former.

According to Basu *et al.* [1936] green gram (*Phaseolus mungo* ?) is superior to the lentil as a source of protein. These workers mention the botanical name *Phaseolus mungo* for green gram which is a misnomer. The botanical name for green gram is *Phaseolus radiatus*. However considering various factors as shown above it appears that green gram (*Phaseolus radiatus*) and lentils (*Lens esculenta*) appear to be equally good. Niyogi *et al.* [1932] observe that proteins of *Lens esculenta* are highly digestible, while those of *Vigna catjang* have a high biological value. Adolph and Chiang [1935] isolated five proteins of which glutelin has a high content of cystine and the albumin and two globulins are high in lysine.

Swaminathan's [1937, 2] interesting work consisted of comparing the biological values of proteins of pulses combined with rice and also with rice and skimmed milk powder, for maintenance measured by balance sheet method, with that of the biological value for growth and maintenance assessed by growth method. These are shown in Table VII.

According to him, of all the pulses given with raw milled rice, green gram is the best ; next comes red gram and then Bengal gram, soya bean and black gram follow in order of merit. Similarly green gram stands first in its value for maintenance and growth when given in combination with raw milled rice and skimmed milk powder, then Bengal gram, black gram, red gram, and soya bean, in spite of the latter's very high protein content. It is almost useless as a source of protein for growth and maintenance to the rice-eating people.

Regarding the mineral content, green gram (*Phaseolus radiatus*) and lentil are almost identical so far as calcium and phosphorus are concerned, but the iron content of the former is four times higher than that of the latter. The cow gram (*Vigna catjang*) is very deficient in calcium, and all the more richer in phosphorus with a mean iron value between the first and the second. As to the vitamin contents, lentil is very rich, green gram moderately so and cow gram rather poor in vitamin A (carotene), lentil has got less amount of vitamin B_2 than the other two, while lentil and green gram are the same regarding vitamin B_1 content. Taking all these facts into consideration green gram appears to be slightly better than the other two, because of its very high iron content. Lentil is comparatively better than the cow gram, in view of its higher vitamin A as well as B contents.

So far as protein value of soya bean (*Glycine hispida*) is concerned, it appears to be not better than the pulses like *Phaseolus radiatus*, *Lens esculenta* and *Vigna catjang*, on the other hand it is definitely inferior to *Cicer arietinum* and *Phaseolus mungo*. This is quite contrary to the findings of Osborne and Mendel [1917] who in their original studies concluded that the soya bean protein reacted like animal protein. But considering the high fat (19·5 per cent), as well as phosphorus (0·689 per cent) and iron (11·50 mgs. per cent) contents, its total food value is definitely much higher than that of any other pulse. This food value has been further enhanced by the presence of vitamin B_1 which is almost three times as much as vitamin B_2 as dried milk powder and also vitamin B_2 two-thirds as much [Wan, 1932]. According to Miller [1936] " compared with most green vegetables it is unusually rich in good protein, fat, calcium, iron and phosphorus and it is a very good source of vitamins A, B and G." Not much work has been done in India on this pulse which is not very commonly grown in Indian soil. The question is whether, in view of the above findings, extensive cultivation of this pulse should be advocated or not, to improve the nutrition of the people in general. Some of the advantages have just been mentioned but there are some disadvantages too, which may be mentioned below :—

(1) People are not accustomed to take soya bean,

(2) It does not possess a very agreeable taste and is therefore not commonly used as *dhal*,

(3) The soya bean preparations require some skill and are only learnt with some difficulty,

(4) Its value as a source of available protein was over-estimated. This has been proved so by analytical results.

These disadvantages considerably outweigh the advantages of its value as a food in this country. On the other hand it appears that either Bengal gram or pulses of the *Phaseolus* group which are better as a source of protein and are rich in mineral and vitamin contents may prove better than soya bean, if taken with some type of vegetable fat. It will therefore do well

to popularise the indigenous pulses like Bengal gram, black gram, green gram and lentils, than to introduce a new bean the merits of which are not well established.

Conclusions

(1) So far as the nutritive value of the pulses alone is concerned, Bengal gram (*Cicer arietinum*) and black gram (*Phaseolus mungo*) stand first in the list and are alike in mineral and vitamin contents.

(2) Greem gram (*Phaseolus radiatus*) and lentil (*Lens esculenta*) come next in the order of merit. Cow gram (*Vigna catjang*), although it appears to be equally good, does not come to the same level as the other two crops, in respect of the mineral and vitamin contents.

(3) Soya bean as a member of the second group has got a high food value, not as a source of available protein but because of its high fat, mineral and vitamin contents. So there is no special advantage in advocating its cultivation in preference to other indigenous pulses like Bengal gram, black gram, green gram and lentils.

(4) When pulses are eaten with rice, green gram (*Phaseolus radiatus*) and red gram (*Cajanus indicus*) appear to act better than Bengal gram or any other variety and when they are eaten with rice and milk or milk products, green gram (*Phaseolus radiatus*) is the best, the next best being Bengal gram (*Cicer arietinum*).

Summary

Bengal gram has proved to be the best of all the pulses. Green gram is also very good especially when combined with rice or rice and milk products. Black gram has high nutritive value as a protein. Other pulses such as lentil or red gram may be taken only occasionally. Introduction of soya bean is not necessary in this country.

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TABLE I

Digestibility coefficient, biological value and net protein value, at 10 per cent level of intake of different pulses

No.	Pulses	Botanical names	Digestibility per cent			Biological value per cent			Net protein value			Total protein value		
			Niyogi and co-workers	Basu and co-workers	Swami-nathan	Niyogi *et al.*	Basu *et al.*	Swami-nathan	Niyogi *et al.*	Basu *et al.*	Swami-nathan	Niyogi *et al.*	Basu *et al.*	Swami-nathan
1	Bengal gram	*Cicer arietinum*	76	...	86	78	...	62	16·68	...	11·78	28·14	...	21·68
2	Green gram	*Phaseolus radiatus*	83	90	86	60	52	51	13·92	10·4	10·44	25·25	23·26	24·77
3	Red gram	*Cajanus indicus*	71	...	75	74	...	85, 72	13·47	...	12·23	25·63	...	23·57
4	Field pea	*Pisum sativum*	70	70	...	62	48	...	11·10	11·7	7·67	25·57	27·1	24·63
5	Lentil	*Lens esculenta*	78	90	88	58	32	41	12·86	6·5	9·27	28·74	22·6	24·11
6	Khesari	*Lathyrus sativa*	...	...	...	...	41	...	...	...	...	...	32·2	...
7	Black gram	*Phaseolus mungo*	82	...	78	64	...	62	13·81	...	11·36	25·00	...	23·50
8	Cow gram	*Vigna catjang*	58	...	78	72	...	45	10·86	...	8·47	26·52	...	24·12
9	Field bean	*Dolichos lablab*	65	...	76	57	...	41	10·65	...	7·67	28·74	...	24·63
10	Soya bean	*Glycine hispida*	...	90	76	...	58	54	...	20·2	16·42	...	41·2	42·41
11	Horse gram	*Dolichos biflorus*	59	...	73	67	...	66	10·43	...	10·65	28·4	...	22·12

TABLE II

Amino-acids of the globulin of pulses expressed as per cent of protein ash and moisture free

(*Niyogi and co-workers*)

	Bengal gram	Green gram	Red gram	Field pea	Lentil	Black gram	Cow gram	Field bean	Horse gram
Lysine	7.42	7.7	7.03	8.19	6.70	6.16	5.96	7.27	8.15
Histidine	1.42	1.46	2.56	1.90	2.77	3.46	2.21	1.38	2.38
Arginine	10.29	7.90	5.84	9.50	10.35	6.37	7.45	8.09	6.00
Arginine	11.85	8.80	6.91	10.64	11.53	7.17	7.91	8.72	7.07
Cystine	2.02	1.59	1.58	1.12	1.62	3.51	1.89	1.54	1.24
Tyrosine	2.95	3.53	3.12	1.98	3.56	3.63	3.74	4.34	4.01
Tyrosine	4.90	3.92	3.35	2.32	3.92	3.87	3.88	5.11	5.74
Tryptophane . . .	0.46	0.59	0.46	0.51	0.71	0.80	0.80	0.44	0.76
Tryptophane . . .	0.46	0.74	..	..	0.62	0.84	0.59	0.42	1.02

TABLE III

Amino-acids of the glutelin of pulses expressed as per cent of total nitrogen
(Basu and associates, 1937)

	Green gram (*Phaseolus mungo*)	Lentils (*Lens esculenta*)
Arginine	4.88	9.33
Histidine	5.81	0.96
Cystine	1.30	0.84
Lysine	4.99	10.44
Tyrosine	3.30	3.97
Tryptophane	0.52	0.59

TABLE IV

Amino-acids of the proteins of khesari (Lathyrus sativa) *precipitated at different saturations, expressed as the percentage of total nitrogen*
(Basu and co-workers, 1937)

	Precipitated by 3/10 ammonium sulphate	Precipitated by 1/2 ammonium sulphate	Precipitated by ammonium sulphate
Arginine	5.74	3.93	10.45
Histidine	2.83	1.45	8.24
Cystine	0.71	0.53	0.25
Lysine	7.35	12.83	10.75
Tyrosine	3.67	2.91	3.38
Tryptophane	0.65	Trace	0.74

TABLE V

Position in order of merit from various stand-points

Basis of consideration	1	2	3	4	5
Total protein content	Soya bean	Field bean	Lentil	Bengal gram	Cow gram
Biological value	Red gram	Bengal gram	Horse gram	Black gram	Cow gram
Digestibility	Lentil	Green gram	Soya bean	Bengal gram	Black gram
Net protein value	Soya bean	Bengal gram	Red gram	Black gram	Green gram
Essential amino-acids—					
Cystine	Black gram	Bengal gram	Cow gram	Lentil	Green gram
Tyrosine	Soya bean	Field bean	Bengal gram	Cow gram	Lentil
Tryptophane	Soya bean	Black gram	Cow gram	Khesari	Green gram
Histidine	Khesari	Black gram	Lentil	Red gram	Horse gram

TABLE VI

Respective merits of different pulses

—	Total protein	Biological value	Digestibility	Net protein	Cystine	Tyrosine	Tryptophane	Histidine	Total
	1	2	2	1	1	1	1	1	10
Bengal gram	1	2	2	1	..	1	1	..	8
Green gram	..	..	2	1	1	1	..	1	6
Red gram	..	2	..	1	1	..	..	..	4
Field pea	1	2	..	..	..	..	..	..	3
Lentil	1	..	2	..	1	1	1	..	6
Khesari	..	..	..	..	1	..	..	1	2
Black gram	..	2	2	1	1	1	..	1	8
Cow gram	1	2	..	..	..	1	1	1	6
Field bean	1	..	..	..	..	..	1	..	2
Soya bean	1	..	2	1	..	1	1	0	6
Horse gram	..	2	..	..	..	..	1	1	4

TABLE VII

Biological values of proteins of pulses combined with rice and also with rice and skimmed milk powder

Sources of protein in the diets at 8 per cent level of intake	Biological value for maintenance measured by balance sheet method	Digestive coefficient	Biological value for growth and maintenance by growth method	Average increase in body weight (*g*)
Series 1				
Raw milled rice+Skimmed milk powder.	80	89	1·86	12·6
Raw milled rice+Red gram .	70	81	1·26	7·4
Raw milled rice+Soya bean .	68	84	1·13	6·5
Raw milled rice+Bengal gram	66	85	1·21	6·8
Raw milled rice+Black gram.	61	82	1·12	5·9
Raw milled rice+Green gram.	59	90	1·29	7·5
Series 2				
Raw milled rice+Red gram +Skimmed milk powder.	90	87	1·49	8·3
Raw milled rice+Soya bean +Skimmed milk powder.	86	93	1·33	7·3
Raw milled rice+Bengal gram +Skimmed milk powder.	80	92	1·39	9·9
Raw milled rice+Black gram +Skimmed milk powder.	79	96	1·89	8·3
Raw milled rice+Green gram +Skimmed milk powder.	77	95	2·04	10·9

NOTES

NOTIFICATION No. F. 46-27/38, DATED THE 13TH OCTOBER 1938, OF THE GOVERNMENT OF INDIA IN THE DEPARTMENT OF EDUCATION, HEALTH AND LANDS

THE following Plant Quarantine Regulations issued by the Government of Ceylon under the Ceylon Plant Protection Ordinance, No. 10 of 1924, which came into force in that country from the 1st October, 1938, are published for general information :—

THE PLANT PROTECTION ORDINANCE, NO. 10 OF 1924

Regulation made by the Governor by virtue of the powers vested in him by section 9 of the Plant Protection Ordinance, No. 10 of 1924, and by Article 93 of the Ceylon (State Council) Order in Council, 1931.

B. H. ALUWIHARE,
Acting Minister for Agriculture and Lands

Colombo, July 22, 1938

REGULATION

The regulations set forth in the Schedule to the Plant Protection Ordinance, No. 10 of 1924, as last amended by regulation published in *Gazette* No. 8276 of March 12, 1937, are hereby further amended with effect from the 30th day of September, 1938, by the substitution, for the regulations contained in Part I of that Schedule, of the following new regulations :—

" 1. (1) No person shall import any plant into Ceylon through any port other than the port of Colombo or the port of Talaimannar : Provided that any of the following articles may be imported through any port, namely, onions, gingelly seed, dried turmeric, tamarind, " Karanai " roots, ground nuts, paddy, gram, dhal and other pulses, dried chillies, curry stuffs, or other culinary seed.

(2) No person shall import or land a living specimen of any insect or invertebrate animal not already known to exist in Ceylon without the written permission, previously obtained of the Director of Agriculture : Provided that the Director of Agriculture or the Director, Colombo Museum, may make such importations as may be required for scientific investigations.

2. *Prohibitions and Restrictions.*—No person shall import or land—

(*a*) any seed or plant of any species of *Hevea* grown in the Western Hemisphere ;

(*b*) except under a permit in writing from the Director of Agriculture, any seed or plant or any species of *Hevea* grown in the Eastern Hemisphere ;

(*c*) any tea seed, directly or indirectly, from any place in India, except in such quantities as may be necessary for experimental purposes and except under and in accordance with the terms and conditions of a permit in writing from the Director of Agriculture ;

(*d*) any coconut plant ;

(*e*) any coconut in husk, through any port other than the port of Colombo ;

(*f*) the seed of cotton of any species of *Gossypium*, through any port other than the port of Colombo ;

(*g*) any seed, or unginned cotton or raw ginned cotton of any species of *Gossypium* grown in the Western Hemisphere, through any port other than the port of Colombo or from any port other than Bombay, or except with a certificate of fumigation or disinfection or both fumigation and disinfection from the Collector of Customs at Bombay ;

(*h*) except under a permit in writing from the Director of Agriculture, any living part of the cassava plant, *Manihot utilissima.*

3. (1) No person shall import any fresh fruit into Ceylon without a licence issued by the Director of Agriculture, or by an officer of the Department of Agriculture authorised in that behalf by the Director, or otherwise than in accordance with the terms and conditions of such licence.

(2) No licence shall be issued for the import of fresh fruit from any country in which the Mediterranean Fruit Fly (*Ceratitis capitata*) or any other pest of fruits which has not become established in Ceylon is known or suspected to exist unless the Director of Agriculture is satisfied that either before export from such country or during transit to Ceylon the fruit is so treated as to ensure that such pest, in all its stages, is effectively destroyed.

(3) Every licence issued under this regulation shall—

(*a*) specify the country from which the fresh fruit may be imported ;

(*b*) be valid for the period specified in the licence ;

(*c*) be subject to the condition that it may be withdrawn by the Director of Agriculture at any time prior to the expiry of that period by written notice addressed to the holder of the licence ;

(*d*) be subject to such other special conditions as may be set out in such licence by or by the authority of the Director of Agriculture ; and

(*e*) be in the form set out hereunder :—

"THE PLANT PROTECTION ORDINANCE, NO. 10 OF 1924

Licence to Import Fresh Fruit

Under regulation 3 of the regulations set forth in Part I of the Schedule to the Plant Protection Ordinance, No. 10 of 1924, as amended by regulation published in *Gazette* No. ———— of ————, ———— (name) ———— of ———— (place) ———— is hereby licensed to import the undermentioned varieties of fresh fruit from—

———————————————

———————————————

———————————————

———————————————

This licence expires on ————————————————

This licence is subject to the condition that it may be withdrawn by Director of Agriculture at any time prior to the date set out above by written notice addressed to the holder of this licence.

This licence is further subject to the special conditions set out overleaf.

————————,

(for) *Director of Agriculture*

———— (date) ———— "

(4) No person shall land any fresh fruit at any port other than the port of Colombo, and no person shall land such fruit at Colombo, unless he has satisfied the Principal Collector of Customs that such fruit was grown in and consigned from the country specified in the licence issued to that person.

4. *Inspection, Fumigation, and Disinfection.*—(1) All living imported plants, coconuts in husk, and the seed of all species of *Gossypium*, shall, before passing out of the Customs, be examined by the Inspector in charge of the Colombo Fumigatorium, together with the packings, cases, pots or coverings in which they may be packed : Provided that this paragraph shall not apply to potatoes, onions, turmeric, culinary seed, edible vegetables, and the seed of such vegetables and the seed of any ornamental plant.

(2) Every imported plant, which on inspection is found or suspected to be infected with any pest or disease, shall be subjected to fumigation or disinfection, or both fumigation and disinfection, as may be deemed necessary by the Inspector : Provided that the Director of Agriculture, by notification published in the *Gazette*, may direct, from time to time, that all imported plants of any species or genus, or all plants of any specified species or genus imported from any specified part of the world, shall be subjected to

fumigation or disinfection or both fumigation and disinfection; and after the publication of any such notification all consignments of the plants referred to therein shall be subjected to fumigation or disinfection or both fumigation and disinfection, as the case may be.

(3) Where fumigation or disinfection is necessary, such fumigation or disinfection shall be carried out at the Colombo Fumigatorium.

(4) The Director of Agriculture may order that any plant which has been fumigated or disinfected shall be grown for a period of quarantine in special nurseries provided or approved by him for that purpose.

(5) Every plant which is to be fumigated or disinfected under this regulation shall, after it is landed at the Customs premises, be conveyed to the Fumigatorium under Customs supervision at the expense of the consignee or his agent.

(6) As soon as possible after the receipt of any imported plant or package of imported plants at the Fumigatorium such plant or package shall be examined by the Inspector and fumigated or disinfected or both fumigated and disinfected, as the circumstances may require.

(7) After examination and any necessary treatment, the articles shall be delivered to the consignee together with a certificate showing that they have been dealt with as required by this regulation.

(8) No person shall remove any article from the Fumigatorium until he has obtained the certificate referred to in paragraph (7).

(9) The certificate referred to in paragraph (7) shall not be granted until all fees due in respect of supervision and fumigation have been paid in such manner as the Principal Collector of Customs may direct.

(10) Fumigation or disinfection shall be carried out at the risk of the consignee, and the consignee or his agent shall be in attendance during the process of unpacking the articles for inspection, fumigation, or disinfection and of repacking them thereafter, and shall provide the labour necessary for handling the articles during such processes.

(11) Where any plants are sent to Ceylon through the Post Office from a place beyond Ceylon the plants shall be conveyed to the Fumigatorium under the supervision of the Postal Authorities at the expense of the consignee or his agent and shall be examined by the Inspector and dealt with as prescribed in the foregoing paragraphs.

(12) The consignee shall pay in respect of each plant or package conveyed to the Fumigatorium—

(*a*) where it is conveyed under the supervision of the Customs Authorities, a supervision fee of Re. 1 for any period from 9 A.M. to 4 P.M., together with a further fee calculated at the rate of 25 cents per hour for any period between 4 P.M. and 9 P.M.; and

(*b*) where it is conveyed under the supervision of the Postal Authorities, a supervision fee of 50 cents for any period from 9 A.M. to 4 P.M., together with a further fee calculated at the rate of 25 cents per hour for any period between 4 P.M. and 9 P.M.

(13) The consignee shall, in addition to the fee specified in paragraph (12), pay a fee of 25 cents per package to cover the cost of fumigation or disinfection.

(14) Any imported plant which in the opinion of the Inspector cannot be cleaned by fumigation or other treatment shall, together with the packings, cases, pots or covering in which it may be packed, be destroyed at the expense of the consignee."

A LIST OF INTERNATIONAL CONGRESSES AND CONFERENCES TO BE HELD DURING THE NEXT FIVE YEARS

Name	Place	Date	Organising Body or the Secretary
1. VIIth International Congress of Genetics.	Edinburgh .	August 1939.	Prof. F. A. E. Crew, Institute of Animal Genetics, University of Edinburgh.
2. VIIth International Botanical Congress.	Stockholm . .	1940 . .	Dr. R. Florin, Riksmuseum, Stockholm 50, Sweden.
3. Congress of the International Society of Soil Science.	Germany . .	July 1940 .	Prof. F. Schucht, Invalidenstrasse 42, Berlin N. 4.
4. International Congress on Entomology.	(Not yet decided, probably Holland.)	1941 . .	..
5. Imperial Entomological Conference.	(Not yet decided)	1940 or 1941 (Not yet decided).	..
6. VIIIth International Congress of Tropical and Sub-Tropical Agriculture.	Tripoli . .	13th to 17th March 1939.	Under the auspices of the Ministry of Italian Africa. The President of the Executive Committee, VIIIth International Congress of Tropical and Sub-Tropical Agriculture, F. I. T. A., *via* Regina, Elena 86, Rome, Italy.
7. XVIIIth International Congress of Agriculture.	Dresden (Germany)	6-12th June 1939.	Dr. F. Sohn, Berlin S. W. 11, Hafenplatz 4.

NEW VICE-PRESIDENT OF THE INTERNATIONAL INSTITUTE OF AGRICULTURE, ROME

THE Permanent Committee of the International Institute of Agriculture has unanimously elected Mr. J. Clyde Marquis, Delegate of the United States Government, as Vice-President of the Institute in succession to Dr. J. J. L. van Rijn, Delegate of the Netherlands Government, whose three-year term of office has just expired.

RECENT PUBLICATIONS OF THE IMPERIAL AGRICULTURAL BUREAUX

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Annual subscription (postage paid) (Back numbers still available)	40	0

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Index Veterinarius. First issue April, 1933. Annual subscription (postage paid). Volumes I to III mimeographed, Volume IV onwards printed (Vols. I—V issued quarterly. Vol. VI onward issued half yearly) (Back numbers still available).	100	0

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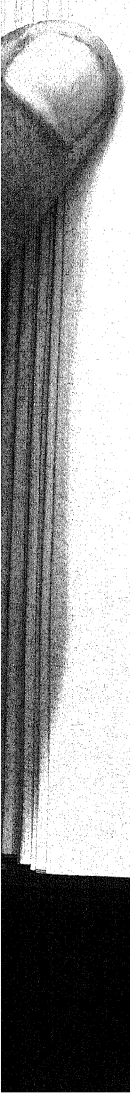

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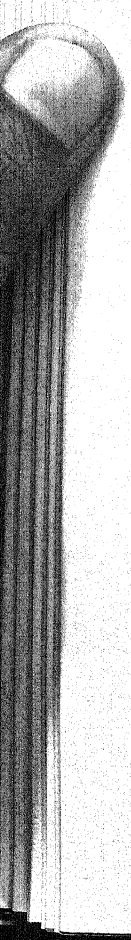

Imperial Council of Agricultural Research
Instructions to Authors*

1. All manuscripts should be clean, clear and carefully revised. Only one side of the paper should be used. The original type-written copy and not a carbon copy should be sent. Capitals should be sparingly used, and all the necessary punctuations should be done in the MS. and not left over for introduction in proofs. Particular attention should be devoted to the following points :—

2. *Spelling.*—Errors creep into typescript and are apt to be missed in a cursory reading. The scientific names of plants and insects require special attention. The combinations 'upto' and 'uptil' are incorrect and should not be used.

3. *Use of capitals.*—(See also paragraphs 12 and 13). A simple rule is to avoid these except when they *must* be used. At present manuscripts require a good deal of correction, usually in the elimination of unnecessary capitals.

4. *Punctuation.*—A simple rule to follow is 'when in doubt, punctuate'. Most manuscripts suffer more from lack of commas than from their presence.

5. Referees have repeatedly drawn attention to various other faults some of which are as follows :—

(i) Careless arrangement of tabular matter,

(ii) Unnecessarily voluminous appendices,

(iii) Diffuseness in the text.

6. It ought to be kept in mind that research work is judged largely on the clearness of the published account. The reduction of a large amount of research data to a clear and readable paper *cannot* be accomplished at the first writing. In the first draft the author is generally arranging his own material and ideas and is writing or dictating with a view to get down every thing in some kind of order and with no eye either to the best disposition of the material or the clearest statement of the results. A second write-up is absolutely essential and in some cases it is worthwhile doing a third. The result will justify the labour spent.

7. The title of a paper should not be lengthy.

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8. It is desirable that MS. should have suitable heads and sub-heads. In numbering the principal divisions of a paper, Roman numerals should be used. The use of Arabic figures and (*a*), (*b*), (*c*), etc., is generally reserved for numbering the sub-divisions coming under each head.

9. Articles submitted for publication either in the *Indian Journal of Agricultural Science* or in the *Indian Journal of Veterinary Science and Animal Husbandry* should be accompanied by abstracts for publication in *Agriculture and Live-stock in India*. Abstracts should be concise, but should be long enough to explain the matter dealt with; ordinarily no abstract should exceed 200 words.

10. When a word or line is intended to be printed in *italics* it should be underlined with a single line, in SMALL CAPITALS with two lines, in CAPITALS with three lines, and when in **antique** (heavy type) with a wavy line (~~~~).

11. In descriptive matter, numbers under 100 and all numbers occurring at the beginning of a sentence should be in words.

12. Local names for crops, technical operations, etc., should be defined where they first occur in the text, *e.g.*, *rabi* (spring crop). The use of local weights and measures should be avoided as far as possible. Vernacular names, such as *jowar*, *bajri*, should be in italics without a capital letter, and each such name where it first occurs should be followed by its scientific equivalent in brackets, *e.g.*, *jowar* (*Andropogon Sorghum*). It is usual to write the initial letters of varietal names in capitals, *e.g.*, Striped Mauritius, Dharwar-American cotton and Broach cotton.

13. Botanical and zoological names are printed in italics and should be underlined in the MS., *e.g.*, *Triticum vulgare* L.; *Diplodia Corchori* Syd.; *Pyrilla aberrans* Kirby. The International Rules of Botanical Nomenclature and the International Rules of Zoological Nomenclature should be followed. The names of chemical substances should not be written with a capital letter.

14. The following and similar abbreviations may be used freely—*viz.*, *e.g.*, *i.e.*, mm. (millimetre), cm. (centimetre), grm. (gramme), mg. (milligramme), c.c. (cubic centimetre), sp. gr. (specific gravity), lb. (pound), cwt. (hundredweight), in. (inch), ft. (foot), oz. (ounce), md. (maund), sr. (seer). Other abbreviations should be used sparingly, if at all.

15. References to plates should be given within brackets, without prefixing the word " see " or " cf." in the MS. itself, and should not be left over for introduction in proofs. For example, " The parasite (Plate X, fig. 4) was present late in 1906 ".

16. The word " Table " is preferable to " Statement ", and tables should be numbered consecutively in Roman figures. Each table should have an explanation as a sub-head. It is more convenient for reference if tables can

be printed horizontally; for this purpose they should not exceed in width the printing measure of the page (5 in.). *Example*—

TABLE IV

Results of water-saving experiments on wheat (Pusa 12) at Gungapur, Haripur and Sargodha, 1916-17

Station	No. of irrigations including the preliminary watering	Yield per acre in maunds and seers		Average yield per acre	
		Grain	Straw	Grain	Straw
		Mds. Srs.	Mds. Srs.	Mds. Srs.	Mds. Srs.
Gungapur .	One	12 19½	20 10		
Haripur . .	,,	8 31	19 14	9 34	21 17
Sargodha . .	,,	8 12½	25 27½		

17. References to literature, arranged alphabetically according to authors' names, should be placed at the end of the article, the various references to each author being arranged chronologically. Each reference should contain the name of the author (with initials), the year of publication, the abbreviated title of the publication, volume and page. In the text, the reference should be indicated by the author's name, followed by the year of publication enclosed in brackets; when the author's name occurs in the text, the year of publication only need be given in brackets. If reference is made to several articles published by one author in a single year, these should be numbered in sequence and the number quoted after year both in the text and in the collected references. This system of referencing is the same as is used in the *Biochemical Journal* with slight modification and will be clear from the following illustration:—

> The work of Osborne and Mendel [1919, 1, 2] and Steenbock and Boutwell [1919] had indicated an association of the fat-soluble vitamin with the green parts of plants. This view was examined by Coward and Drummond [1921] who reported that vitamin A was not synthesised by etiolated shoots but that green leaves were active in its formation. Another worker [Wilson, 1922], on the other hand, found that etiolated shoots if given in sufficient quantity could supply the fat-soluble vitamin and that this factor was therefore formed in the absence of light.

REFERENCES

Coward, K. H. and Drummond, J. C. (1921). *Biochem. J.* **15**, 530

Osborne, T. B. and Mendel, L. B. (1919, 1). *J. Biol. Chem.* **37**, 187

——————————— (1919, 2). *J. Biol. Chem.* **41**, 549

Steenbock, H. and Boutwell, R. (1919). *J. Biol. Chem.* **41**, 149

Wilson, J. (1922). *J. Biol. Chem.* **51**, 455

Abbreviations, as far as possible, should follow the system adopted in " A World List of Scientific Periodicals " published by the Oxford University Press.

18. Sources of information should be specifically acknowledged, so that readers may distinguish between the contributions to knowledge of the author and of others.

19. Papers should be complete when submitted for publication. As alterations and additions at the proof stage cause both additional expense and delay, they should be resorted to as little as possible. In making corrections in proofs, the recognized symbols which will be found in the " Standard Dictionary " should be used. Second (page) proofs will be submitted to authors who should return them promptly.

Illustrations

20. As the *format* of the journals has been standardized, the size adopted being crown quarto (about 7 1/8 in. × 9 5/8 in. cut), no text-figure, when printed, should exceed 4 1/2 × 5 inches. Figures for plates should be so planned as to fill a crown quarto plate—the maximum space available for figures being 5 3/4 × 8 inches exclusive of that for letterpress printing.

21. Photos or drawings for illustration should accompany the manuscript and each should bear on the reverse side the name of the paper to which it relates together with the title or legend, figure or plate number, and the size to be reproduced. When giving instructions for reduction, linear measurements are understood; thus, " half-size " means reduce to half the length and breadth, not half the area. A photograph should not be rolled up, nor pinned, and should always be packed flat. A complete list of plates and figures should always accompany the paper.

22. Line drawings should be made with clear black lines on smooth white paper, preferably Bristol board. Rough paper should be avoided. Care should be taken that all the lines are drawn firmly; scratchy or grey lines, produced by the ink being thinned down, are not permissible. Drawings should be larger than the required size. All lettering should be neatly and clearly put in, care being taken to make all lettering sufficiently large to stand reduction. The lettering used in the graphs of *The Economist* is specially commended to the notice of authors.

23. For half-tone work, copy should be made on glossy silver paper and of the same size or larger than the size required. Any photographs sent should be clear, properly exposed and with suitable contrast between the various parts of the picture. Every photograph should contain in itself something that will give the scale of the objects photographed. Where accuracy is important, this can be a tape or a foot-rule or a marked pole, but where accuracy is unimportant and merely the proportions of things have to be indicated, such indicators as a match-box, a book or the figure of a man (according to the size of the objects photographed) are sufficient.

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25. For detailed instructions regarding preparation of illustrations, it would be of advantage to refer to Mr. C. M. Hutchinson's article on " Photographic illustrations " in the *Agricultural Journal of India*, Vol. XI, Pt. 3, July 1916, and Mr. A. W. Slater's paper on " The Preparation and Reproduction of Scientific Illustrations " in the *Proceedings of the Third Entomological Meeting*, 1919, which has been reprinted as *Bulletin No. 114 of the Agricultural Research Institute, Pusa.*

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ORIGINAL ARTICLES

THE GENUS *FUSARIUM*

I. KNOWN OCCURRENCE IN INDIA

BY

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(Received for publication on 12th September 1938)

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I. Introduction

Amongst the fungi there is probably no single genus which causes more havoc in cultivated crops than the genus *Fusarium*. In India two or three highly important diseases due to species of *Fusarium* are known. Amongst these, most work has been done on the wilt diseases of cotton and pigeon-pea, especially as regards the search for resistant varieties. It may be anticipated that in time similar work will be done on resistance in the case of other diseases due to *Fusarium*.

There is one point which has to be kept clearly in mind before any attempts are made at testing varieties of crops for resistance to disease. It is quite futile for any worker to set out to look for resistance unless he first has a good understanding whether there exist several species of *Fusarium*, and possibly several forms of a species, capable of attacking different groups of varieties of the host. There are cases known in India where more than one distinct species of *Fusarium* is capable of causing a disease. Thus for instance, it will be seen later that dry rot of potatoes (*Solanum tuberosum*) may be due to *Fusarium oxysporum*, *F. coeruleum*, *F. javanicum* v. *radicicola*, and *F. trichothecioides* and that at least two *Fusaria* are concerned with a disease of

pigeon-pea (*Cajanus cajan=C. indicus*). Experiments at Delhi have indicated that several other *Fusarium* diseases of crop plants are similarly complicated.

It therefore becomes a primary concern of the plant pathologist first of all to learn the species of *Fusarium* with which he is working, and if possible to study even the physiologic forms, before he commences on elaborate system of testing varieties for resistance.

The best way to study such a complicated genus as *Fusarium* is to have workers making a special study of the genus at large, and not restrict them to one single species. With the object of opening the way for such a study the occurrence of the genus in India known to date is reviewed here.

II. Species recorded in India

The species recorded in India have been listed in Table I. In arranging the groups within the table the authority used is Wollenweber and Reinking [1935]. The groups are listed in column 1 in the same order as Wollenweber and Reinking have used in their key, and in column 2 the fungi are listed according to the names now used by those workers. In column 3 are the names given by the authors, to whom reference is made in column 7. Columns 4, 5 and 6 give the habitat, locality, and type of disease, if any.

At the present time only six of Wollenweber and Reinking's sixteen groups are represented, though five of the six sub-groups of Elegans and Discolor are found. Only fifteen of the known sixty-five species have so far been recorded, or if varieties and forms are included, two dozen out of the known twelve dozen. Not all these fungi have definitely been related to diseases, the diseases established by experiment as being due to *Fusaria* in India being as follows :—

Top-rot of sugarcane
Foot-rot of rice
Fruit-rot of oranges
Dry-rot of potato tubers
Foot-rot of pigeon-pea
Wilt of flax
Wilt of plantain
Wilt of pigeon-pea
Wilt of cotton
Wilt of *til*
Wilt of sann-hemp
Wilt of potatoes

TABLE I

Species of Fusarium *recorded in India*

Section	Name accepted by Wollenweber and Reinking	Name given by authors	Host or habitat	Locality	Type of disease caused	Reference
Sporotrichiella .	...	...	...	...	...	.....
Liseola .	*F. moniliforme* Sheld .	*F. moniliforme* Sheld .	Sugarcane (*Saccharum*)	Various parts of India.	Top rot . .	McRae, W. [1930, 1934].
	Ditto . .	*F. moniliforme* Sheld. var. *majus* Wr. et Rg.	Rice (*Oryza sativa* L.)	Madras Presidency.	Foot rot . .	Sundararaman, S. [1935].
	Ditto . .	*F. moniliforme* Sheld.	Apple (*Pyrus malus*) .	United Provious.	?	Mitter, J. H. [1935].
	Ditto . .	Ditto . .	Dead leaf sheath of Sorghum (*Andropogon sorghum*).	?	...	Mitra, A. [1935].
	Ditto . .	Ditto . .	Fruits of orange (*Citrus cryocarpa*).	Bengal . .	Storage rot of fruit.	Ghatak, P. N. [1938].
Spicarioides . .	...	...	...	...	...	.....
Elegans (Orthocera)	*F. lini* Bolley . .	*F. lini* Bolley . .	Linseed (*Linum usitatissimum*).	Pusa (Bihar) .	Wilt . .	Mitra, M. [1925].
	Ditto . .	Ditto . .	Ditto . .	Do. .	Do. .	McRae, W. [1926].
	Ditto . .	Ditto . .	Ditto . .	Unstated. .	...	Anonymous [1925].
	Ditto . .	Ditto . .	Ditto . .	Bombay Presidency.	...	Uppal, B. N., Patel, M. K. and Kamat, M. N. [1935].
(Constrictum) .	*F. bulbigenum* Cke. et Mass. *v. lycopersici* (Brushi) Wr. et Rg.	*F. lycopersici* Sacc. .	Tomato (*Lycopersicum esculentum*)	Do. .	...	Ditto.
(Oxysporum) .	*F. oxysporum* Schl. .	*F. oxysporum* Schl. .	Potato (*Solanum tuberosum*).	Do. .	Dry rot of tubers.	Uppal, B. N. [1929].
	Ditto . .	Ditto . .	Ditto . .	Do. .	...	Uppal, B. N., Patel, M. K. and Kamat, M. N. [1935].
	Ditto . .	Ditto . .	Ditto . .	Do. .	Dry rot of tubers.	Nagpurkar, S. D. and Kulkarni, G. S. [1921].
	F. oxysporum Schl. *v. nicotianae* John.	*F. oxysporum* Schl. var. *nicotianae* Johnson.	Tobacco (*Nicotiana tabacum*).	Do. .	...	Uppal, B. N., Patel, M. K. and Kamat, M. N. [1935].
	F. oxysporum Schl. *v. cubense* (E. F. Sm.) Wr. et Rg.	*F. cubense* E. F. Sm. .	Plantain (*Musa* sp.) .	Bengal . .	Wilt . .	Hector, G. P. [1927].

TABLE I—*contd.*

Section	Name accepted by Wollenweber and Reinking	Name given by authors	Host or habitat	Locality	Type of disease caused	Reference
	F. oxysporum Schl. *v. cubense* (E. F. Sm.) Wr. et Rg.	*F. cubense* E. F. Sm. *v. inodoratum* Brandes.	Plantain (*Musa* sp.) .	Madras Presidency.	Wilt . .	Sundararaman, S. [1930].
	Ditto . .	*F. oxysporum* Schl. f. 3 Wr.	Plantain (*Musa sapientum*).	Poona (Bombay Presidency).	...	Uppal, B. N., Patel, M. K. and Kamat, M. N. [1935].
	? *F. vasinfectum* Atk. *forma*.	*F. udum* Butler . .	Pigeon-pea (*Cajanus cajan*=*C. indicus*).	Various parts of India.	Wilt . .	Butler, E. J. [1910].
	Ditto . .	Ditto . .	Ditto . .	Berar . .	Do. .	Pearl, R. T. [1923].
	F. vasinfectum Atk. .	?	Cotton (*Gossypium* sp.)	Various parts of India.	Do. .	Shaw, F. J. F. [1914].
	? Ditto . .	?	Ditto . .	Central Provinces.	Do. .	Ajrekar, S. L. and Bal, D. V. [1921].
(Oxysporum) .	? *F. vasinfectum* Atk. .	*F. udum* Butler . .	Cotton (*Gossypium* sp.)	Do. .	Do. .	Ajrekar, S. L. [1926].
	Ditto . .	Ditto . .	(*Sesamum*) . .	...	Wilt . .	Butler, E. J. [1926].
	F. vasinfectum Atk. .	*F. vasinfectum* Atk .	Sann-hemp (*Crotalaria juncea*).	...	Do. .	Ditto.
	Ditto .	Ditto . .	Ditto . .	Pusa (Bihar) .	Do. .	Mitra, M. [1934].
Martiella . .	*F. coeruleum* (Lib.) Sacc.	*F. coeruleum* (Lib.) Sacc.	Potato (*Solanum tuberosum*).	Pusa (Bihar) and Bombay.	Do. .	Uppal, B. N. and Kulkarni N. T. [1937].
	Ditto .	Ditto . .	Ditto . .	Bombay Presidency.	Dry rot . .	Ajrekar, S. L. and Kamat, M. N. [1923].
	Ditto . .	Ditto . .	Ditto . .	Do. .	Do. .	Uppal, B. N. [1929].
	F. solani (Mart.) App. et Wr.	*F. viride* (*F. solani v. medium* Wr.)	Ditto . .	Bombay Presidency (common).	Do. .	Uppal, B. N., Patel, M. K. and Kamat, M. N. [1935].
	Ditto . .	*F. viride* (Lechm.) Wr. renamed as *F. solani* var *medium* Wr.	Pigeon-pea (*Cajanus cajan*=*C. indicus*).	United Provinces.	?	Mitter, J. H. [1935].
	F. javanicum Koord *v. radicicola* Wr.	*F. radicicola* Wr. .	Potato (*Solanum tuberosum*).	?	...	Mitra, A. [1935].
	Ditto . .	Ditto . .	Ditto . .	Bombay Presidency.	Wilt . .	Ajrekar, S. L. and Kamat, M. N. [1923].
	Ditto . .	Ditto . .	Ditto . .	Do. .	Khoka (dry) rot	Uppal, B. N. [1929].
			Ditto . .	Bombay Presidency (common).	Wilt and dry rot	Uppal, B. N., Patel, M. K. and Kamat, M. N. [1935].

Eupionnotes	...	...	...	...	...	.....
Pseudomicrocera	...	...	...	...	...	.....
Submicrocera	...	...	...	...	...	.....
Arachnites	...	...	...	...	...	.....
Macroconia	...	...	...	...	...	.....
Lateritium	*F. lateritium* Nees	*F. limonis* Briosi	Lemon. (*Citrus medica* var *limonis*).	Allahabad (U. P.).	?	Mitter, J.H. and Tandon, R. N. [1930].
	F. lateritium Nees var. *uncinatum* Wr.	*F. uncinatum* Wr.	Pigeon-pea (*Cajanus cajan* = *C. indicus*).	Pusa (Bihar)	?	Wollenweber, H. W. [1917].
	Ditto	*F. lateritium* Nees. var *uncinatum* Wr.	(This is the same isolate as above).	Do.	Foot rot	Wollenweber, H. W. [1938].
Arthrosporiella	*F. camptoceras* Wr. et Rg.	*F. camptoceras* Wr. et Rg.	Bajra (*Pennisetum typhoideum*. Isolated from the ear.	?	...	Mitra, A. [1935].
	F. semitectum Berk. et Rav.	*F. semitectum* Berk. et Rav.	Fruit of *Citrus medica*	?	...	Ditto.
	F. diversisporium Sherb.	*F. diversisporium* Sherb.	Petiole of a wilted leaf of *Carica papaya*.	?	..	Ditto.
	F. semitectum Berk. et Rav. v. *majus* Wr.	*F. incarnatum* (Rob.) Sacc. renamed as *F. semitectum* Berk. et Rav. v. *majus* Wr.	Shoot of *Aegle marmelos*.	?	...	Ditto.
Gibbosum	...	...	...	...	...	...
Discolor (Trichothecioides).	*F. trichothecioides* Wr..	*F. trichothecioides* Wr..	Potato (*Solanum tuberosum*).	Bombay Presidency.	Dry rot	Nagpurkar, S. D. and Kulkarni, G. S. [1921].
	Ditto	Ditto	Ditto	Do.	Do.	Uppal, B. N. [1929].
	Ditto	Ditto	Ditto	Bombay Presidency (common).	Do.	Uppal, B. N., Patel, M. K. and Kamat, M. N. [1935].
(Neesiola)	...	...	...	...	...	.....
(Saubinettii)	*F. sambucinum* Fuck	*F. pannosum* Massee	Living trunks of *Cornus macrophylla*.	Punjab	?	Butler, E. J. and Bisby, G. R. [1931].
	Ditto	*F. roseum* Lk.	Roots and stems of *Zinnia elegans*.	Bombay Presidency.	?	Uppal, B. N. Patel, M. K. and Kamat, M. N. [1935].
	F. culmorum (W. G. Sm.).	*F. culmorum* (W. G. Sm.) Sacc. v. *leteius* Sherb.	Wheat (*Triticum vulgare*).	Do.	...	Ditto.
Ventricosum	...	...	...	...	...	.....

In addition, one or two other diseases, such as wilt of tobacco, head-blight of wheat and fruit spotting of citrus, may reasonably be expected to have been caused by the species of *Fusarium* isolated.

Thus it is seen that seven out of the twelve definitely established *Fusarium* diseases in India are wilt diseases. Six of these seven wilts are caused by representatives of the group Elegans.

If we consider for a moment the names given by the authors and compare them with names finally adopted by Wollenweber and Reinking [1935] we are struck by the number of changes that have been made. In spite of the fact that most of the work has been done fairly recently, out of the twenty-four species, varieties and forms mentioned above, thirteen names have been changed by Wollenweber and Reinking. This means that Wollenweber and Reinking have considered these names, which have been given first by workers elsewhere, to be merely synonyms of other species of *Fusarium*.

In addition to the above there have been reports of unidentified species of *Fusarium* in India. Narasimhan [1929] described a species of *Fusarium* which causes wilt of gram (*Cicer arietinum*) but unfortunately his description is not sufficiently detailed for identification purposes. Uppal, Patel and Kamat [1935] report species of *Fusarium* on *Arachis hypogaea, Cicer arietinum* and *Lathyrus sativus* in Bombay Presidency. Mitter and Tandon [1930] report unidentified species of *Fusarium* at Allahabad on *Capparis sepiara, Cassia obtusifolia, Artocarpus integrifolia, Sorghum vulgare, Cucurbita moschata,* and *Pennisetum typhoideum.*

Mitra [1931] has reported isolations of *Fusarium* from cinchona. McRae [1924] obtained twelve isolates of *Fusarium* from wilted plants of gram (*Cicer arietinum*) but repeated attempts to infect gram with these isolates met with failure [Mitra, 1925].

III. Difficulties in the way of identification

It has already been pointed out that it is fundamentally essential to get correct identifications. This is needed for the guidance of the plant pathologist himself and for those who read his published work. If the importance of this is not realised from the changes in names shown in Table I, it will at any rate be obvious from an example which will be discussed here and which gives some indication of the types of difficulties met with and some clues as to how these difficulties may be overcome.

In the year 1910 after a thorough investigation of the wilt-disease of pigeon-pea (*Cajanus cajan* = *C. indicus*) Butler [1910] described a fungus which he called *Fusarium udum* and which caused wilt. The name was given, as he said, without prejudice to the question whether or not it had been previously described under a different name. Later he [Butler, 1926] reached the conclusion that it could not be distinguished from *Fusarium vasinfectum*, which attacks also cotton and sesamum. In fact he was of the opinion that it

could perhaps not be distinguished even from *F. oxysporum v. cubense* (=*F. cubense*), a previously described species of the Section Elegans. Meanwhile a large amount of work was being done by McRae and Shaw, which was published as a monograph in 1933 [McRae and Shaw, 1933] on resistance of pigeon-pea to *F. vasinfectum*. Mitra [1934] worked on the *Fusarium vasinfectum* attacking sann-hemp and concluded that it could attack also pigeon-pea but not cotton. Uppal and Kulkarni [1937] however failed to repeat the findings of Mitra in that they found that *F. vasinfectum* from sann-hemp could not infect pigeon-pea or *vice versa* though they could infect their own hosts heavily. These authors raised an objection to the method used by Mitra of inoculating with wilted material of sann-hemp or pigeon-pea of the previous year's growth.

Wollenweber [1938] published results of experiments in which he isolated from pigeon-pea material sent by Butler from Pusa in 1905 yet another parasitic *Fusarium*, which causes not a true wilt but a foot-rot and which he called *F. lateritium v. uncinatum*. He isolated the fungus in 1911 not from vascular tissue but from very small sporodochia typically present on the infected roots and stem base of the plants. He again listed *F. udum* Butl. under its old name and not under the name *F. vasinfectum*.

When all these published results are considered two things now become clear :—

1. It is possible that several species of *Fusarium* may be found associated with a wilted crop like pigeon-pea.

2. The differences between isolates several of which may cause wilt of a crop and all of which may be regarded as belonging to one species may show a considerable range of variation in both morphological characters and ability to cause infection, and it is highly important to study this question.

With regard to the first point it is clear that the mere isolation of a wilt-causing organism, and its proof as a cause of wilt by laboratory experiments, must not lead to a hasty conclusion that it is the organism commonly attributed to the disease or the only one which is concerned. That can only be determined by a careful study of the fungus.

The second point raises the whole question of the value of different characters used in identification of species of *Fusarium* and it will be pertinent here to consider the general trend of thought on the species concept in relation to mycological taxonomy and *Fusarium* in particular. The identification of species of *Fusarium* today is based on a mass of data acquired by a number of workers during the past forty years. This work has been brought together in a book by Wollenweber and Reinking [1935]. These workers use in their classification morphological and physiological characters more or less indiscriminately, though tending to stress the morphological, after growing on 'standard' media prepared from plant products. Such characters as size and shape of spores are given somewhat more weight than colour of the stroma and discoloration of medium and similar characters. Some workers have

revolted against this system. Thus Hansford [1925], for instance, speaking of the Section Elegans concluded that " the present classification of the section is useless and that our present conception of a species of *Fusarium* must be revised and broadened." He was of the opinion that the size of macroconidia, the presence of sporodochia, the colour of mycelium, substratum and sporodochia, and the presence and colour of sclerotia, were highly variable and of little value for classificatory purposes. He preferred to regard all the fungi which he studied in the Section Elegans as strains of a single species. His views were supported by Butler [1929], who, enlarging on the case of *Fusarium vasinfectum*, wrote as follows :—

" In Egypt, Fahmy (according to letters received from him) has under study three strains of cotton-wilt producing fungi of the *F. vasinfectum* type : one from America which mostly attacks American varieties ; one from India restricted to Indian cottons ; and one from Egypt chiefly on the Egyptian. Morphological differences between the three strains are very slight, but there are cultural differences. If specialization of parasitism is the guide, there would seem to be as good reason for giving specific rank to the Indian strain, which refuses to attack the Egpytian cottons, as to most other species in the Elegans Section, but what is to be done with the Egyptian strain which some times attacks the Indian varieties ? " Again he says of the value of host resistance as compared with morphologic characters for species delimitation, " Hosts that are resistant under one set of conditions may become susceptible under another. Even a variation of temperature may sometimes break down resistance to infection as shown at Madison and elsewhere, and it must not be forgotten that for a parasite the host plant constitutes a large part of its environment. " In concluding he says, " If we admit that there is no such thing in nature as a pure, invariable, homogeneous species, that habit can modify the forms of a species so long as it endures, that morphological characters are less readily modified by the environment than physiological, and that convenience is often the best guide where to draw the line between different species, we may get some degree of guidance as to which of the tendencies defined above may best be followed. We will be led, I think, on the whole to prefer the morphological basis of classification and only in special cases to stress biological differences ".

The opinions of Butler were expressed at the International Congress of Plant Sciences held at Ithaca in 1926. The remarks were provoked partly by those workers, including Gaümann studying *Peronospora*, who were tending to create large numbers of new species based on host relations. His views were those of most speakers on the subject at the conference. For instance, Shear [1929, 1] based his main criticism of parasitism as a means of species delimitation on the grounds that it is rather readily acquired by fungi and that parasites and saprophytes frequently occur in the same genus. " For practical purposes therefore it appears necessary to use the term species for groups of individuals which can be separated by recognizable morphological characters, admitting that in many cases these groups are somewhat arbitrarily established and that there are intergrading forms with other species ". This, it is presumed, meets with the requirement which he expressed in another paper

[Shear ; 1929, 2] that " The essential purpose and function of nomenclature, as I understand it, is to provide a reasonably stable, convenient, uniform and accurate means of designating groups of living organisms ". Cunningham [1929] complains that the complicated nature of the technic involved in the delimitation of species by cultural characters would lead (and has led) to this work being limited to a few specialists, a defect that would become more serious as time passes. The case of the genus *Fusarium* is picked out by him as the horrible example—" For, thanks to those few pathologists who have worked with this genus, it is in such a chaotic condition that the writer believes few mycologists or pathologists would undertake the delimitation of any species placed therein ". He believes that the solution lies in more precise monographing of species of genera occurring in any one region, describing and arranging the species according to morphological characters.

Stakman [1923] considers that physiologic characters are of equal value with morphological ones in delimiting species. In his experience with *Puccinia graminis* he found the physiological character of host specialization quite as fixed and stable as any morphological characteristic. He pointed out that most of the work done on bridging hosts was invalidated by the fact that the workers used mixed physiologic forms, so that the so-called bridging host phenomenon can no longer be held up as proof of the instability of host reactions. " If we are seeking the ultimate truth regarding fungi, surely we ought to accept it in plant behaviour as well as in plant structure ". He emphasised the importance, however, of using a pure line of the host.

Important contributions have recently come from Michigan, and their revolutionary ideas need careful consideration. Coons and Strong [1931] worked out a system of identifying a large number of *Fusaria* by their reaction to certain aniline dyes and copper compounds in artificial culture media. The important characteristics used after growing the cultures on the standard media were colours of mycelium, breadth of mycelial mat, changes in colours of medium, type of edge of the colony, and growth form. There were two colours of mycelium, red and white (a third has since been added, brown) ; various sizes of the colony ; four categories under change in medium colour ; three types of colony edge ; and nine different ' growth forms ' of mycelium. Based on these differences a key for the large number of *Fusaria* studied was prepared. According to these workers there was a " striking parallelism in the independent classification of the cultures by a *Fusarium* specialist (Sherbakoff) and the dye reactions ". Hansford [1926], Brown [1928], Mitter [1929] and others had criticised the present basis of classification of *Fusaria* on grounds of high variability within the ' species ', and Coons and Strong said " If the assignment, as done by Sherbakoff, of members of the Elegans Section into species were unreliable, to take only this section as an example, and resulted in divergent forms such as *F. cubense* and *F. oxysporum*, and others of the Elegans Section being ' not satisfactorily divided up ' as Hansford states is the true situation when present methods of classification are attempted, it would seem that contradictions in the dye response would have been inevitable ".

With this publication, then, there did not seem to be much doubt about the point of view of the Michigan workers. Whatever they might do in the way of providing a simple key by which workers in the genus might readily get a clue as to the identity of the organisms they were working with, they considered that on the whole the views of Sherbakoff on classification, and therefore inconsequence those of Wollenweber and others to whose school Sherbakoff belonged, were acceptable and that the fears of Hansford, Brown, Mitter, Butler and others were unfounded. What was wanted was not a new classification, but a short-cut to identification. Names of species would presumably remain unaltered.

The second paper from Michigan, that by Nelson, Coons and Cochran [1937] dashes to the ground any hopes, to which the first paper might have given birth, that the present classification was to be stabilised. No longer is the old system upheld. In fact, " it is extremely doubtful if this system is workable when applied to the separation of pathogenic and saprophytic *Fusaria* in the sub-section Orthocera ". Hansford's views, previously rejected, are now thrown into the front line of attack, for he is stated to have shown that *Fusarium cubense* is extremely variable morphologically and to have criticised sharply the elaborate system of classification in use. It is not mentioned whether Brown and Mitter are brought back into favour in like manner, but the admission of the unreliability of morphological characters would presuppose this. *Fusarium orthoceras* var. *apii* Wall and Rg. is no more. *Fusarium apii* and *F. apii* var. *pallidum* now rise up, distinguished by their host relationships.

We read " The authors believe that the celerypathogenes differ sufficiently from other members of the Orthocera group to constitute a new species. The most important differential character is the distinct host relationship and it is chiefly on this basis that the segregation is made ". Yet of the two varieties it is said " Because of their morphological similarities, these pathogenes are best considered as belonging to one species ". These two statements clearly mean.—

1. That these two fungi are species separate from all other species of *Fusarium* because of their host reaction.

2. That although in their host reactions the two fungi are somewhat different, they belong to the same species because they are morphologically alike !

Such is the present state of confusion in the genus *Fusarium*. It is fashionable to point out the wretched genus as one of the black sheep in a bad family. It is fashionable to demonstrate how variable is the genus *Fusarium*, and to criticise Wollenweber's classification, yet his help in identification is keenly sought. For after all he has reduced 1100 or so *Fusaria* to the very modest total of 144 including varieties or forms, he has prepared a synonymy, and the pathologist and mycologist alike are eternally grateful.

It seems that if we are to make still more improvements, we must think of the genus *Fusarium* in terms of related groups and of systematics in general. The prevalent idea of a species is well expressed by Gates [1938]. He classifies the criteria of a Linnean species into morphological differences in the phenotype and its chromosome number and their morphology, physiological and chemical differences, and differences in ecology and geographical distribution. He agrees that all Linneons need not answer all these requirements, but insists that " In the practical work of describing and dealing with species the only essential, however, is a sufficient number of morphological differences." If we are to treat *Fusarium* on a similar level with higher plants and with other fungi, our first essential for species delimitation is " a sufficient number of morphological differences ".

There can be no doubt about the high degree of variability of certain morphological characters, including those such as spore size and septation. A study of this type of variation is being made by Mme Raillo [1935]. But the view that certain physiological characters are less variable seems to be at present hardly acceptable. Samuel and Greaney [1937] recently showed that wide variation existed in the extent to which thirteen isolates of *F. culmorum* collected from wheat fields in England caused root-rot of wheat in controlled experiments, the average disease rating varying from nine to forty. The writer has further observed that many cultures of *Fusarium culmorum* and *F. avenaceum* have a wide host range. It may be that in the Section Elegans specialization is of a higher order, but even if this is proved it remains essential that throughout the genus any particular type of character should have the same general significance of rank. Host range cannot be taken for species value in the case of sections Discolor and Roseum, and it seems discordant to elevate it to specific rank in the case of the Section Elegans.

It is sometimes held that the only use for systematics is to find a pigeon hole bearing a name for the plant under consideration, that it is a mere tool. This is a confusion of ideas. The whole Linnean system, to which the plant pathologist still clings, is based, imperfect though it may be, on the idea of relationship in its broadest sense. A systematic arrangement is an entirely different thing from a key, as seemed at first to be realized by Coons and Strong. If we can rapidly form the opinion that a certain fungus is *Fusarium orthoceras* var. *apii* or *F. apii* or whatever it may be called because it attacks celery, this short-cut is very convenient. Much time is saved by such a device, and confirmation of the identity by studying other characters is greatly simplified. But this has nothing to do with systematics.

One cannot form opinions on the taxonomy of an organism without studying all closely related forms. Yet the worker in Northern Europe cannot compare the pathogenicity of *Fusarium* species of the Section Elegans on, say, bananas or cotton. Even in the crops which can be grown in various countries pathogenicity is closely bound up with variable factors such as temperature and moisture, as has been clearly shown by workers on various diseases at Wisconsin, and soil conditions as shown by Mundkur [1936] working with

cotton wilt in India. The delimitation of species on the basis of host reaction demands the use of a pure line of the host and a definition of the conditions—probably an impossibility.

One can only conclude that in the end it will be necessary to base specific rank upon characters easily recognizable under standardized conditions available in all moderately equipped mycological laboratories. This precludes the use of host relationships, which must be given a rank lower than species. In the meantime practical problems have to be solved and until something better arises, there seems nothing for it but to try to stick to Wollenweber's system. Inadequate though it may be, there is nothing better. The creation of new specific names at least should be done with the greatest caution until an acceptable alternative is constructed.

IV. Summary

(1) A primary consideration in selecting varieties of crops resistant to diseases caused by species of *Fusarium* is the determination of the organism or organisms responsible.

(2) The species of *Fusarium* recorded in India are listed. Twenty-four species or varieties are found belonging to six groups. Twelve diseases have been definitely proved to be caused by *Fusarium* and seven of these are wilt diseases. Six of these seven wilts are caused by *Fusarium* species of the Section Elegans.

(3) The importance of correct identification, and some of the reasons why this is difficult, is brought out by consideration of the case of the *Fusarium* species attacking pigeon-pea (*Cajanus cajan*), cotton (*Gossypium* spp.), *til* (*Sesamum indicum*) and sann-hemp (*Crotalaria juncea*).

(4) The value of physiologic characters such as host relationships in the species concept is discussed with special reference to *Fusarium*.

(5) It should be the ultimate aim to improve the classification of the genus *Fusarium* but in the meantime it is desirable to accept that of Wollenweber and his associates, creating as few new species as possible, and avoiding the use of any new biological characters, such as host reaction, for species delimitation.

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STUDIES IN INDIAN CHILLIES

(5) INHERITANCE OF ANTHER COLOUR AND ITS RELATION TO COLOUR IN PETAL AND NODE IN *CAPSICUM ANNUUM* L.*

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I. Introduction

The most common colour in anthers of Indian types of chillies is either blue or purple with occasional streaks or patches of yellow. The intensity and extent of colour may, however, vary in different types.

In order to study the mode of inheritance of anther colour, the Pusa Types 3 and 29 [Shaw and Khan, 1928]—the former having bluish purple anthers and the latter bluish with yellow streaks—were crossed with a yellow-anthered mutant, T 11A.

While the F_2 of the cross T 3 × T 11A was being studied it was observed that the purple or blue anthers were associated with purplish nodes and the yellow anthers with green nodes. Though the original idea was to study only the inheritance of colour in anther, complete observations on a part of the F_2 population with regard to colour in anther, petal and node being available, it is possible to bring out interesting facts regarding the relationships between anther colour and colour in petal and node.

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II. Experimental results

Cross T 3 × T 11A

T 3 has bluish purple anthers, purple petals and purple nodes and T 11A has yellow anthers, white petals and green nodes. In F_1, the anthers and nodes were light purple and the petals partially purple, *i.e.*, white with purple streaks. In F_2, a good deal of variation was observed in these characters, and particularly in the anther colour, suggesting that they were controlled by more than one factor. The plants with coloured anthers, whatever the intensity or extent of colour, had invariably purple nodes. The petal colour, however, segregated independently. The following frequencies were observed in F_2 :—

	Purple anthers and purple nodes (various grades)	Yellow anthers and green nodes	Total
T 3 × T 11A	252	78	330
T 11A × T 3	330	87	417
Total observed	582	165	747
Total expected on 3 : 1 basis .	560·25	186·75	747

$\chi^2 = 3{\cdot}39$; P between 0·1 and 0·05. The fit is fair.

Anther and node colour frequencies in relation to petal colour

	Anthers and nodes purple		Anthers yellow, nodes green	Total
	Petal purple	Petal white	Petal white	
Total observed . . .	133	24	41	198
Total expected on 9 : 3 : 4 basis	111·6	37·2	49·6	198·4

$\chi^2 = 10{\cdot}4$; P less than 0·01

The fit is bad. The bad fit and the absence of cultures breeding pure for ' white petals and purple anthers and nodes ' in F_3 necessitated the examination in detail of the segregations in the thirteen F_3 cultures behaving like F_2. The segregations and the values of χ^2 are given below :—

F_3 culture No.	Anthers and nodes purple		Anthers yellow, nodes green	Total	χ^2
	Petal purple	Petal white	Petal white		
1	25	4	5	34	4·14
2	27	3	16	46	5·30
1—18	32	4	6	42	6·81
1—25	29	4	7	40	4·40
1—45	27	4	8	39	2·98
1—55	22	5	12	39	1·24
1—76	31	5	9	45	3·13
1—142	30	4	8	42	3·30
1—205	26	3	10	39	4·23
2—23	26	5	13	44	2·12
2—98	28	4	8	40	3·07
2—106	28	4	7	39	3·95
2—113	26	5	5	36	3·87
Total .	357	54	114	525	Very high

' P ' being higher than 0·05 in all cases except in culture No. 1-18, the fits are good. The fit for the total of all the cultures is very poor. This is due to the phenotype ' white petals, purple anthers and nodes ' being consistently deficient.

The striking thing about these segregations is that in all the thirteen families the phenotype ' white petals and purple anthers and nodes' is deficient.

This points out that some lethal factor is operating which also may be responsible for the absence of cultures breeding pure for ' white petals and purple anthers and nodes '.

In F_3 the various cultures showed the following behaviour :—

No. of cultures in F_3 and nature of parent plant in F_2	F_3 behaviour	Formulae	Frequencies	
			Observed	Expected
11 cultures from plants with deep purple petals, anthers and nodes	Pure	**SSAABB SSAABb SSAAbb**	11	11
27 cultures from plan s with petals, anthers, and nodes like F_1 in colour	Petals, anthers and nodes purple / White petals, anthers and nodes purple. 3 : 1	**SSAaBB SSAaBb**	3	6·75
	Petals, anthers and nodes purple. / White petals, yellow anthers and green nodes. 3 : 1	**SsAABB SsAABb**	11	6·75
	Like F_2 . . .	**SsAaBB SsAaBb**	13	13·50
11 cultures from plants with white petals, and purple anthers and nodes	Pure . . . . / White petals, and purple anthers and nodes / White petals, and yellow anthers and green nodes 3 : 1	**SSaaBB SsaaBB SsaaBb**	0 / 11	3·66 / 7·32
7 cultures from plants with white petals, yellow anthers and green nodes	Pure	**ssaaBB ssaaBb ssaabb**	7	7

The results are fairly satisfactory. The absence of cultures breeding pure for white petals with purple anthers and nodes may be due to the combination, homozygous **S** and **B**, being lethal as has been pointed out earlier.

Cross T 29 × T 11A

The second cross studied in this connection was between Types 29 and 11A. Both the parents have white petals, and there is thus no segregation for petal colour. T 29 has purple colour in nodes and anthers, but the anthers are neither so completely nor so deeply purple as in T 3. The yellow colour shows itself at places, particularly towards the base. T 11A has yellow anthers and green nodes. In F_1 the anthers were yellow with light purple tips. The nodes were light purple. In F_2 no plant with yellow anthers (like T 11A) and purple nodes, or *vice versa*, was observed. The following were the observed frequencies in F_2 :—

	Anthers and nodes purple like T 29	Anthers and nodes purple like F_1	Anthers yellow and nodes green like T 11A	Total
Total observed . . .	157	298	177	632
Total expected on 1 : 2 : 1 basis	158	316	158	632

$\chi^2 = 3 \cdot 30$; P between $0 \cdot 2$ and $0 \cdot 1$. The fit is good.

In F_3 the following segregations were observed :—

No. of cultures in F_3 and nature of parent plant in F_2	Segregations	Formulae	Frequencies	
			Observed	Expected
6 cultures from plants with anthers and nodes like Type 29	Pure . .	SSaabb .	6	6
9 cultures from plants with anthers and nodes like F_1 .	Like F_2 .	Ssaabb .	9	9
1 culture from plants with yellow anthers and green nodes (T 11A)	Pure . .	ssaabb .	1	1

The F_3 results are in agreement with the F_2 observations. The recessive nature of yellow anthers and green nodes having been established in the first cross, only one F_2 plant like Type 11A was carried to the F_3.

III. Theory

In order to explain the foregoing results it is necesary to assume that :—

S=a factor for the production of purple colour in the plant except in petals. It also converts yellow anthers to yellowish purple and green nodes to purple. When **S** is absent, no purple colour will manifest itself even when other colour factors are present.

A=a factor which produces purple colour in petals if **S** is present, *i.e.*, **S** and **A** are complementary for colour in petals.

B=a factor which intensifies purple colour in the presence of **A**.

The factorial constitution of the parents and the F_1's is as follows :—

Type 3	**AABBSS**
Type 29	**aabbSS**
Type 11A	**aabbss**
T 3 × 11A,F_1	**AaBbSs**
T 29×11A,F_1	**aabbSs**

IV. Application of theory to observed results

With regard to the colour in anthers, petals and nodes in the cross, Type 3×Type 11A, since Type 3 is homozygous for the presence of **S**, **A**, and **B**, and Type 11A lacks all these, the F_1 is heterozygous for all the three factors and has purple petals, anthers, and nodes. In F_2 the segregation of these three factors should result as follows :—

SAB	**SAb**	**SaB**	**Sab**	**sAB**	**sAb**	**saB**	**sab**
27	**9**	**9**	**3**	**9**	**3**	**3**	**1**
Pr. petals, anthers and nodes		White petals, pr. anthers and nodes		White petals, yellow anthers and green nodes			

	36	: 12	:	16
or	9	: 3	:	4

This is actually observed in F_2 (page 186).

Considering the plants with purple anthers and nodes against those with yellow anthers and green nodes, a 3 : 1 ratio is expected which is realized in F_2.

The genotypes of the above phenotypes with their F_3 behaviour are given below :—

Genotypes of 27 **SAB**	Behaviour in F_3
1 **SSAABB** . .	Breed pure for deep purple petals, anthers and nodes.
2 **SSAABb** . .	
2 **SSAaBB** . .	Segregate into 3 purple petals, anthers and nodes : 1 white petals, purple anthers and nodes.
4 **SSAaBb** . .	
2 **SsAABB** . .	Segregate into 3 purple petals, anthers and nodes : 1 white petals, yellow anthers and green nodes.
4 **SsAABb** . .	
4 **SsAaBB** . .	Like F_2.
8 **SsAaBb** . .	
Genotypes of 9 **SaB**	
1 **SSaaBB**	Breed pure for white petals and purple anthers and nodes.
2 **SSaaBb** . .	
2 **SsaaBB** . .	Segregate into 3 white petals, purple anthers and nodes : 1 white petals, yellow anthers and green nodes.
4 **SsaaBb** . .	

All the other phenotypes will breed true to white petals, yellow anthers and green nodes, as they lack **S**, the basic factor for production of purple colour in plant.

Excepting the failure to realize cultures breeding pure for white petals with purple anthers and nodes, and a slightly bad fit in F_2, the observed results agree with the theory.

In the other cross, T 29 × T 11A, the segregation of **S** should result as follows :—

SS	:	**Ss**	:	**ss**
1		2		1
Like T 29		Like F_1		Like T 11A

This is realized (page 189).

In F_2 the parental types should breed true and the plants like F_1 should segregate like F_2. This also is realized (Page 189).

V. Discussion

The results of both the crosses indicate that yellow colour in anther of Type 11A is recessive to the two types of purple colours present in the anthers of Type 3 and Type 29 respectively, and that the purple colour in anther and that in the node are determined by the same factor, **S**.

A good deal of variation in the itensity and extent of purple colour in the anthers and nodes of F_2 plants of the cross, Type 3 × Type 11A, indicated that the two parents differ in more than one factor. In addition to this the

appearance of plants with white petals and purple anthers and nodes in F_2 suggested further complications in their inheritance. The precise nature of the mode of inheritance of these characters became clear only during the course of the F_3 study. On referring back to the F_2 data and examining the relation of anther and node colour to petal colour, it was observed that at least three factors were concerned in determining the colour in petals, anthers, and nodes in this cross. It was then for the first time observed that a factor, termed **S**, was necessary for producing purple colour in any plant organ in addition to the other colour factors.

The study of the inheritance of the anther and node colour in the cross, T 29 × T 11A, was simple, the results showing a monohybrid segregation. The only factor in which the parents differ in this cross is **S**, which is both the basic factor for purple colour and the factor for colour in anthers and nodes.

In this connection a reference to the results of the cross T 3 × T 29, so far as they refer to the colour in anthers and nodes [Deshpande, 1933], seems necessary. The parents have been already described. The F_1 was practically like the F_1 of T 3 × T 11A, that is, purple (partially) for petals, anthers and nodes. In F_2 the T 29 form of petals, anthers and nodes was recovered as the recessive (one-fourth of the total F_2 population). This phenotype was similar to the phenotype with white petals, purple anthers and nodes realized as three-sixteenths of the F_2 population in the cross T 3 × T 11A in the present study. Moreover the F_2 plants segregating in F_3 into ' plants with purple anthers and nodes ' and ' plants with yellow anthers and green nodes ' resembled the F_1 of the cross T 29 × T 11A. Thus a relationship in these three crosses so far as the colour in petals, anthers and nodes is concerned, has been traced.

On the evidence furnished by the results of the present investigations, necessary modifications have been made in the genetic formulae previously proposed for types 3 and 29 [Deshpande, 1933]. The original results, however, are not affected by this modification.

VI. Summary

1. It is observed that the manifestation of purple colour in any organ in the plant is dependent upon the presence of a factor, **S**.

2. Yellow anthers of T 11A are recessive to the yellowish purple anthers of T 29 and to deep purple anthers of T 3 on a 1 : 3 ratio.

3. Segregation of anther and node colour in relation to petal colour was found to be on a 36 : 12 : 16 trihybrid ratio.

4. The factor **S** determines the purple colour both in anthers and nodes.

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STUDIES ON QUALITY IN RICE

III. *IN VITRO* DIGESTIBILITIES OF DIFFERENT VARIETIES OF RICE

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It is well known that starches of different cereals are generally distinguishable from one another by the size, shape and optical properties of their granules. It has been shown that they may also be differentiated by their unequal resistance towards amylolytic enzymes and in possessing different liquefaction or gelatinisation temperatures. Among the numerous publications showing the differential effects of amylases upon starches from various sources such as wheat, barley, maize, rice, potato, etc., mention may be made of the researches of Stone [1896], O'Sullivan [1904], Wollf and Fernbach [1907], Hammerstein [1908], Reinitzer [1909], Lang [1910], Nagao [1911], Pauletig [1917], Welzmuller [1921], Giri [1935], and others. The digestibility of the starch from any particular source would also naturally depend on its method of preparation, extent of disintegration, etc., although very little systematic work would appear to have been done on this subject.

Rice is particularly rich in starch and there are a very large number of varieties in existence. Although in experiments on the digestibilities of the starches of rice varieties, the differences to be looked for would not be so marked as those found by experimenters who have compared starches from very different sources, it may yet be probable that the ease of digestion of the carbohydrate and protein constituents in rice may vary with different varieties. Indeed, in practice, there is a general preference exercised by a large section of the consumers for the finer and white varieties of rice compared to the so-called coarser types. Further, there is a belief that polished rice is more easily digested than unpolished rice ; parboiled rice is also generally considered to be more sustaining than raw rice. Scientific study in regard to the digestibility of rice varieties has, however, been wanting.

In two recent communications, Basu and co-workers [1935, 1936] have studied the digestibility by taka-diastase, ptyalin and pancreas-amylase of the starches of different varieties of Bengal rice subjected to different treatments. The present inquiry, which was commenced before the publication of these

papers, relates to the action, by salivary and pancreatic amylases, on different varieties of rice (mostly grown in south India), unpolished as well as polished to varying degrees, the action of bran and of bran constituents and, the effect of parboiling on the digestibility of starch.

Experimental

Materials

Rice varieties.—Different pure strains, most of them grown in the Agricultural Research Stations of the Madras Presidency, were used for the studies. Detailed chemical examination of the varieties have been reported in a previous publication [Sadasivan and Sreenivasan, 1938]. Husking, polishing to different degrees and parboiling were all done in the manner outlined in an earlier paper [Sreenivasan *et al.*, 1938].

Enzymes.—Human saliva, diluted with an equal volume of water was used as the source of ptyalin while, a 0·5 per cent solution of trypsin (Pfansteil), which contains amylase, was employed as the source of the pancreatic amylase. The enzyme solutions were centrifuged and filtered before use. When not in use, they were preserved with toluene in an ice chest. The activities of the enzyme preparations used in the following studies were not the same throughout although, for each particular series of experiments, the same preparations were employed.

Method.—Some preliminary experiments were first carried out studying the action of the enzymes on whole grains of rice cooked according to usual procedure. Difficulty was experienced in obtaining homogeneous samples for estimation from the reaction mixture and highly discordant results were being obtained. Accordingly, samples of the different varieties of shelled rice were powdered and passed through sixty-mesh sieve; requisite quantities were then treated with a little water, boiled for about five minutes and then autoclaved for thirty minutes at 15 lbs. pressure. It was thereby ensured that the different samples were gelatinised completely and under comparable conditions. The autoclaved samples were finally made up to a known volume so as to yield in all cases a final suspension of one per cent starch concentration, the starch in the flours being previously estimated according to A. O. A. C. [1930].

The reaction-mixture consisted of 20 c. c. of phosphate buffer having a *p*H of 6·8, 50 c. c. of starch or rice flour suspension, one c. c. of normal sodium chloride solution and 27 c. c. of water. The mixture was placed in a thermostat at 35 $\pm$ 0·1°C. and, when the contents of the flask had acquired the temperature of the bath, 2 c. c. of the enzyme solution was added, thus making a total volume of 100 c. c. Aliquots (usually 20 c.c.) of the reaction mixture were removed immediately after addition of the enzyme and at intervals of five, ten, and fifteen minutes and the reducing sugars estimated by the method of Willstätter and Schudel [1918]. The results were expressed in terms of mg. of maltose in the total volume of the reaction mixture.

Effect of polishing on the digestibility of raw rice

Three varieties (Adt 11, GEB 24, and Co 9) of rice were polished to different degrees and the digestibility of the unpolished and polished specimens by salivary and pancreatic amylases were studied in the manner outlined above (Tables I*a* and I*b*).

TABLE I*a*

Degree of polishing in relation to digestibility of raw rice by ptyalin

Variety Adt 11				Variety GEB 24				Variety Co 9			
Degree of polishing (per cent)	Mg. maltose formed at end of (mins.)			Degree of polishing (per cent)	Mg. maltose formed at end of (mins.)			Degree of polishing (per cent)	Mg. maltose formed at end of (mins.)		
	5	10	15		5	10	15		5	10	15
Unpolished	155	206	227	Unpolished	227	273	307	Unpolished	244	328	344
6	185	227	244	3	235	319	328	3	252	336	361
10	202	248	260	6	252	332	340	10	239	332	349
16	218	260	277	9	265	353	374	19	235	328	357
22	202	218	269	13	252	336	361				
28	189	218	239	17	260	331	361				

TABLE I*b*

Degree of polishing in relation to digestibility of raw rice by pancreatic amylase

Variety Adt 11				Variety GEB 24				Variety Co 9			
Degree of polishing (per cent)	Mg. maltose formed at end of (mins.)			Degree of polishing (per cent)	Mg. maltose formed at end of (mins.)			Degree of polishing (per cent)	Mg. maltose formed at end of (mins.)		
	5	10	15		5	10	15		5	10	15
Nil	122	197	227	*Nil*	176	269	302	*Nil*	181	260	311
6	134	231	248	3	185	281	315	3	197	281	328
10	147	..	..	6	197	286	315	10	176	269	315
16	143	227	252	9	206	281	256	19	172	248	290
22	126	189	210	13	197	265	281				
28	126	197	239	17	210	290	315				

It may be observed that, in all the varieties, the digestibility increases with polishing, the extent of such increase being different with different varieties. With a very high degree of polishing, there appears, however, to be a slight diminution in the extent of hydrolysis in all the cases. It is probable that, as pointed out later, this is due to the increased viscosity of the cooked rice flour.

More or less similar results are obtained with both the amylases. In view of this and the fact that it was rather difficult to obtain the salivary amylase with nearly the same activity, further studies were carried out with the trypsin preparation alone.

Effect of polishing on the digestibility of parboiled rice

Parboiled rice prepared from two of the varieties (Adt 11 and Co 9) of paddy was polished to three different degrees and the hydrolysis by trypsin-amylase of the different samples was followed as before (Table II).

TABLE II

Degree of polishing in relation to digestibility of parboiled rice by pancreatic amylase

Degree of polishing (per cent)	Variety Adt 11			Variety Co 9		
	Mg. maltose formed at the end of (mins.)			Mg. maltose formed at the end of (mins.)		
	5	10	15	5	10	15
Nil	111	183	221	145	200	247
4	124	205	252	149	217	252
10	136	213	260	158	226	260
15	145	205	247	149	222	273

The results show that, as in raw specimens of rice, the extent of hydrolysis of the rice starch increases with increased polishing while with a high degree of polishing, there is a slight diminution in hydrolysis.

Comparative digestibilities of raw and parboiled rices

In Table III are given the results of comparative digestibilities of raw and parboiled rices from the same varieties (Adt 11 and Co 9), unpolished as well as polished to equal extents (10 per cent).

TABLE III

Digestibility of raw and parboiled rices

Description	Variety Adt 11			Variety Co 9		
	Mg. maltose formed at the end of (mins.)			Mg. maltose formed at the end of (mins.)		
	5	10	15	5	10	15
Raw, unpolished . . .	141	209	247	158	226	235
Parboiled, unpolished .	145	222	269	175	217	256
Raw, polished . . .	171	239	273	217	294	320
Parboiled, polished . .	171	230	264	209	290	337

In the polished specimens, both raw and parboiled rices are digested to about the same extent while with the unpolished specimens, parboiled rice is hydrolysed to a slightly greater extent than the raw one; the difference is however only small. It may be mentioned here that Basu and co-workers [1935, 1936] have reported that parboiling increases the rate of digestion of rice starch in both unpolished and polished specimens.

Viscosity of cooked rice flour

The changes in viscosity of cooked rice flour from raw and parboiled specimens of rice, unpolished and polished, were followed in an Ostwald viscosimeter using the same suspensions that were employed for the above enzymic studies (Tabe IV).

TABLE IV

Changes in viscosity on polishing

	Adt 11—raw rice					
Degree of polishing (per cent) .	0	6	10	16	22	28
Time of flow in viscosimeter (secs.)	122·0	124·5	126·0	129·5	135·0	138·5
	Co 9—parboiled rice					
Degree of polishing (per cent) .	0		4	10	15	
Time of flow in viscosimeter (secs.)	115·5		119·6	126·0	129·5	

The changes in viscosity as a result of parboiling were also studied (Table V.)

TABLE V

Effect of parboiling on viscosity of cooked flour

Variety	Raw—unpolished	Parboiled—unpolished	Raw—polished 10 per cent	Parboiled—polished 10 per cent
	Time of flow in secs.			
Co 9	127·5	115·3	136·0	126·5
Adt 11	122·0	112·5	126·0	118·3

It may be seen from Tables IV and V that with both raw and parboiled specimens, the viscosity increases steadily with increased polishing. Parboiling decreases the viscosity of cooked rice flour from unpolished as well as polished rice; this may be due to the partial dextrinisation of starch during parboiling.

It has been observed in earlier experiments (Tables I and II) that the enzymic digestibility of starch in rice increases with polishing. Since viscosity also increases with polishing and since starch hydrolysis is known to decrease with increased viscosity of the suspension, it would follow that the effect of polishing on the enzymic digestibility will be even more pronounced than has been indicated in these experiments. The slower rate of hydrolysis observed with a very high degree of polishing may be due to the marked increase in viscosity in these cases.

Digestibility of cooked rice grains

It is believed that parboiled rice is more sustaining than raw rice. It is generally consumed only by the labouring class of people and by those with sedantary occupation. Enzymic hydrolysis however shows a slight increase in the digestibility of rice starch on parboiling. Since in these experiments, only the flour (passed through 60-mesh sieve) was used, it was thought that better indications, comparable with conditions obtaining in practice, would be obtained by using cooked whole grains instead of the flour. Accordingly, 10-grms. lots of raw and parboiled rices were taken as whole grains and cooked under similar conditions; the cooked grains were then shaken with a known volume (200 c.c.) of water. To the mixtures were now added, at 35°C., 5 c.c. of a 0·5 per cent solution of pancreatic amylase. Aliquots (20 c.c.) of the reaction mixture were then withdrawn from time to time and the extent of hydrolysis measured as before by estimation of the reducing sugars formed (Table VI).

TABLE VI

Effect of parboiling on digestibility of cooked whole grains

	Mg. maltose formed in total volume of reaction mixture at the end of:		
	10 mins.	20 mins.	30 mins.
Raw, unpolished . .	85	145	333
Parboiled, unpolished . .	60	111	273

It may be noted that the hydrolysis is slower with parboiled rice than with raw rice. This may be due to the fact that parboiled rice yields on cooking a product which is comparatively stiffer in consistency than raw rice and that as a result, there is lesser penetration of the enzyme solution into the cooked grain in the former case.

That the rate of hydrolysis is determined by the extent of penetration of the enzyme into the grain could be seen from the results (Table VII) of the following experiment. A specimen of (raw, polished) rice was cooked in two lots (10 grms. each) under identical conditions. The cooked grains were, in one set of experiments, dispersed in a large volume of water and *M*/15 phosphate buffer (150 c.c. and 50 c.c. respectively) while in the other, they were added in big (rolled) lumps to the same volume of the mixture in another flask. The mixtures were in each case treated with 2 c.c. of 0·5 per cent solution of trypsin and the rates of digestion measured as before.

TABLE VII

Effect of dispersing on the digestibility of cooked whole grains

	Mg. maltose formed in the total volume of reaction-mixture at the end of:		
	10 mins.	20 mins.	30 mins.
Cooked rice grains (dispersed) .	68	119	350
Cooked rice grains (in lumps) .	34	60	85

In the experiments where the cooked grains were added as lumps, the rate of hydrolysis is scarcely half as much as in the other case where the rice was held thoroughly dispersed in the medium.

The above observations were generally confirmed in some *in vivo* trials carried out by one of the authors [Sreenivasan, *unpublished data*].

Digestibility of different varieties of rice

The different varieties of rice (unpolished as well as polished till free from bran layers) whose chemical composition has been reported in the previous paper [Sadasivan and Sreenivasan, 1938] were compared for their digestibility of starch. In a few cases, parboiled specimens of rice were also employed. The activity of the pancreatic preparation was the same throughout this series of experiments and was checked from time to time with a solution of soluble starch of the same concentration. The results are given in Tables VIII *a* and *b*.

TABLE VIII*a*

Digestibility of rice varieties—raw

Variety*		Mg. maltose formed at the end of			Variety		Mg. maltose formed at the end of		
		5 mins.	10 mins.	15 mins.			5 mins.	10 mins.	15 mins.
Co 9	U	132	158	183	Adt 3	U	124	149	183
	P	183	196	226		P	154	218	213
Adt 7	U	98	124	141	T 134	U	98	136	162
	P	154	218	213		P	136	171	179
Co 5	U	77	94	124	T 111	U	119	124	132
	P	166	205	200		P	162	187	213
T 167	U	107	128	132	T 304	U	55	77	85
	P	136	166	196		P	60	85	90
T 149	U	38	55	55	T 356	U	64	68	94
	P	102	132	158		P	90	136	166
Co 1	U	60	90	107	T 59	U	73	85	98
	P	90	102	128		P	107	136	158
T 150	U	98	136	145	T 523	U	43	72	90
	P	115	179	200		P	98	132	141
Co 10	U	81	102	128	GEB 24	U	90	115	141
	P	102	136	158		P	107	132	162
Adt 11	U	51	81	107	K modan	U	153	171	183
	P	94	145	171		P	149	183	200
D modan	U	107	119	136	B modan	U	119	162	174
	P	132	145	162		P	128	166	179
C modan	U	111	141	158					
	P	158	196	226					

*U = unpolished; P = polished (for degree of polishing, *vide* Sadasivan and Sreenivasan, 1938, *loc.-cit.*)

TABLE VIII*b*

Digestibility of rice varieties—parboiled

Variety*		Mg. maltose formed at the end of			Variety		Mg. maltose formed at the end of		
		5 mins.	10 mins.	15 mins.			5 mins.	10 mins.	15 mins.
T 304	U	60	85	94	T 523	U	43	77	90
	P	64	98	90		P	94	136	145
GEB 24	U	98	128	149	T 167	U	102	119	124
	P	107	136	162		P	145	162	183
Adt 3	U	128	154	192	Adt 7	U	102	128	158
	P	149	209	213		P	149	239	252
T 111	U	128	132	136	D modan	U	115	128	145
	P	162	183	213		P	136	145	166
Co 1	U	64	85	111					
	P	94	107	124					

*U = unpolished ; P = polished.

It may be observed that, as in earlier experiments, polished rice is more readily digested than unpolished rice. In the unpolished specimens, parboiled rice is somewhat more digestible than raw rice. There is however a large amount of individual variation between the different varieties. Some rices digest more quickly than others ; again, in some, the rate of digestion is much slower than in others, while, in a few cases, the differences in digestibility between unpolished and polished specimens are very small. The reasons for such large variations are not known but there is no doubt that among the important factors determining the rate and extent of digestibility of the rices, are the nature of the starch and of the bran constituents.

Inhibiting action of rice bran upon starch hydrolysis

Since the foregoing experiments had shown that the digestibility of rice starch increases with the degree of polishing to which the rice is subjected, at any rate under the ordinary conditions of milling, it appeared probable that the bran and outer polishings of the grain may contain some inhibitor of starch hydrolysis by diastases. Further experiments were carried out with a view to studying the nature of this inhibition by the bran and bran constituents.

Effect of bran extract on starch hydrolysis by pancreatin

A specimen of rice polishings obtained from Co 9 after milling the grain to 20 per cent of its weight was autoclaved with five times its weight of water at 15 lbs. for fifteen minutes ; known volumes of the extract were then added to the suspension of (polished) rice gelatinised as before and their effect on the

rate of hydrolysis by pancreatic amylase studied. The results together with the corresponding values for the unpolished and polished specimens of rice are given in Table IX.

TABLE IX

Effect of bran extract on digestibility of polished rice

Substrate	Mg. maltose formed at the end of		
	5 mins.	10 mins.	15 mins.
Co. 9 unpolished rice (control)	145	192	239
,, polished (20 per cent) (control) . . .	166	213	256
,, polished rice + 2 c.c. of autoclaved bran extract	158	207	247
,, polished rice + 4 c.c. of branex tract. .	149	200	239

The results show that the extract of polishings has a definite inhibitory effect on the digestibility of polished rice and that with 4 c.c. of the extract added to the suspension of polished rice, the extent of hydrolysis of the latter becomes about the same as that of the unpolished rice.

In another set of experiments, 5 per cent extracts of bran obtained from both Adt 11 and Co 9, polished to 10 per cent, were employed. Known amounts of the extracts were added to a 2 per cent solution of Zulkowsky's soluble starch prior to hydrolysis by the enzyme (Table X).

TABLE X

Effect of extracts of polishings on starch hydrolysis

Substrate	Polishings from Adt 11			Polishings from Co 9		
	Mg. maltose formed at the end of			Mg. maltose formed at the end of		
	5 mins.	10 mins.	15 mins.	5 mins.	10 mins.	15 mins.
Sol. starch, 2 per cent (control)	64	128	183	64	128	183
Starch + 2 c.c. extract . .	56	119	175	51	98	158
Starch + 4 c.c. extract . .	51	111	162	43	81	137

As before, there is definite inhibition by the bran extracts, the extent of such inhibition increasing with the amount of extract used. The bran from red rice has a greater inhibiting action than that from white rice.

In Table XI are given the results of the comparative effects of polishings (5 per cent extracts) from raw and parboiled rices.

TABLE XI

Effect of raw and parboiled rice polishings on digestion of starch

Substrate	Polishings from Adt 11			Polishings from Co 9		
	Mg. maltose formed at the end of			Mg. maltose formed at the end of		
	5 mins.	10 mins.	15 mins.	5 mins.	10 mins.	15 mins.
Starch—control	124	222	298	124	222	298
Starch + 5 c.c. of raw rice pol. extract	111	209	277	107	200	247
Starch + 5 c.c. of parboiled rice pol. extract	118	213	281	111	209	250

The results show that the effect of the polishings from parboiled rice is definitely less pronounced than that from raw rice.

Action of successive bran layers

The two varieties, Adt 11 and Co 9, both raw and parboiled, were polished to 15 per cent and the polishings collected in three separate fractions, *viz.*, 0 to 5, 5 to 10, and 10 to 15 per cent respectively. From each fraction, a five per cent autoclaved extract was prepared and the inhibitory action of the extracts were studied by adding 5 c.c. of the latter to a 2 per cent solution of Zulkowsky's soluble starch and following the hydrolysis by pancreatin (5 c.c. of 0·5 per cent solution). The results are given in Table XII.

TABLE XII

Inhibiting action on starch hydrolysis by successive bran layers

Substrate	Raw rice						Parboiled rice					
	Adt 11			Co 9			Adt 11			Co 9		
	Mg. maltose formed at the end of (mins.)						Mg. maltose formed at the end of (mins.)					
	5	10	15	5	10	15	5	10	15	5	10	15
Sol. starch—control	171	281	341	171	281	341	166	281	333	166	281	333
Sol. starch + 5 c.c. extract, fr. I	158	264	316	166	260	311	162	273	324	158	273	316
Sol. starch + 5 c.c. extract, fr. II	162	269	324	171	269	328	154	269	324	166	277	324
Sol. starch + 5 c.c. extract, fr. III	166	281	333	171	277	333	158	277	320	166	273	320

It may be seen from Table XII that the inhibiting action is most in the first five per cent of polishings, somewhat less in the second fraction and least in the last fraction ; the difference is less pronounced in the case of polishings obtained from parboiled rice where more or less the same effect is obtained with both the first and second fractions of polishings.

Effect of bran constituents

It has been shown elsewhere [Sreenivasan *et al.*, 1938] that, as a result of polishing of the rice grain, a very large part of the fats, minerals and proteins of the latter are removed with the millings. The inhibitory action of rice polishings on amylolytic digestion may therefore be due to one or more of these constituents. With a view to studying further the nature of the inhibitors, the bran was, in one case, freed from fats by extraction with ether while, in another, it was freed as much as possible from the proteins by successive extraction with 5 per cent sodium chloride solution, the bran being later freed from sodium chloride by washing. Extracts (5 per cent) of the fat-free and nitrogen-free polishings were then prepared and their effect on the hydrolysis of starch by pancreatic amylase (1 c.c. of 0·5 per cent) was compared with that of the original polishings (Table XIII).

TABLE XIII

Effect of fat-free and protein-free polishings on starch hydrolysis

Experiment	Polishings from			
	Adt 11		Co. 9	
	Mg. maltose formed at the end of			
	10 mins.	20 mins.	10 mins.	20 mins.
Soluble starch—control . . .	115	192	115	192
Soluble starch + bran extract—control	102	166	94	158
Soluble starch + bran extract (fat-free)	107	183	102	179
Soluble starch + bran extract (protein-free)	107	183	102	175

It would appear from Table XIII that the inhibitory effect of the bran is due in part to the ether extractives and in part to the proteins.

Effect of extracts of tamarind, chillies, etc., and of ghee

In the following experiments, the effect of addition of small quantities of extracts of tamarind, chillies, etc., and of *ghee* and oil on starch hydrolysis by pancreatic amylase (1 c.c. of 0·5 per cent) was studied. In experiments with

ghee or oil, 2 c.c. of 0·6 per cent calcium chloride was added to the substrate, the latter acting as an emulsifier. The results are recorded in Table XIV. Control experiments were carried out in each case immediately after addition of the enzyme.

TABLE XIV

Effect of addition of tamarind, etc., and of ghee *and oil*

Experiment	Mg. maltose formed at the end of	
	10 mins.	20 mins.
Sol. starch—control	171	252
Sol. starch + 2 c.c. 1 per cent green chillies extract	175	252
Sol. starch + 5 c.c. 1 per cent green chillies extract	175	252
Sol. starch + 2 c.c. 1 per cent red chillies extract	171	252
Sol. starch + 5 c.c. 1 per cent red chillies extract	171	247
Sol. starch + 1 c.c. 1 per cent tamarind extract	171	256
Sol. starch + 3 c.c. 1 per cent tamarind extract	175	252
Sol starch + 1 c.c. 1 per cent tamarind extract (dialysed)	175	256
Sol. starch + 3 c.c. 1 per cent tamarind extract (dialysed)	175	256
Sol. starch + 1 c.c. *ghee*	166	247
Sol. starch + 2 c.c. *ghee*	162	239
Sol. starch + 2 c.c. sesame oil	162	239
Sol. starch + 1 c.c. of *M*/1,000 bile salt	183	264

The results show that extracts of chillies or of tamarind have practically no action while *ghee* or oil has a slight inhibitory effect. Bile salt acts as an activator of starch hydrolysis.

DISCUSSION

The rate of starch hydrolysis in the different varieties of rice, both raw and parboiled, increases with increased polishing until, with a very high degree of polishing, there is no further change in digestibility. There is a large amount of individual variation in the digestibility of the different varieties of rice and, generally speaking, the difference in the digestibility of unpolished and polished rice is greater in red rice and in coarser varieties. These latter contain invariably thicker bran coats than the fine varieties [Sreenivasan and Das Gupta, 1936 ; Ramiah, 1936] and hence, the inhibiting action of bran on starch hydrolysis by diastases should be more pronounced in these cases. The effect of bran decreases with successive polishings so that it would appear that the fats, proteins and mineral constituents of the bran which also decrease in concentration with successive polishings may be responsible for the inhibition. Results of experiments show that such inhibition by individual bran constituents on starch hydrolysis is small though definite. Indeed, the difference in digestibility of unpolished and polished specimens is itself so small as to be insignificant in actual practice. Extracts of tamarind and other spices have

practically no effect on starch hydrolysis, but there is no doubt that, in the animal body, they aid digestion, obviously by stimulating the secretion of the digestive ferments.

Comparative trials with raw and parboiled rices have shown that parboiled rice, especially when unpolished, is more quickly digested by the enzymes than raw rice. This is no doubt due to the partial dextrinization of the starch during parboiling. The experiments however relate to the gelatinized flour from the raw and parboiled rices. With the whole grains, it has been observed that parboiled cooked rice digests much slower than raw cooked rice. Parboiled rice stiffens on cooking and standing whereas raw rice cooks to a soft mass. The former requires mastication and, in consequence, causes a liberal flow of saliva. Under such circumstances, it may be more easily digested than raw rice. When bolted in the same manner as raw rice, parboiled rice may, in fact, be difficult to digest on account of the lesser ease of penetration of the enzymes in such cases. It is likely that the better digestibility of stored rice compared to unstored or fresh rice may also be due to the fact that in the former case, the grains (which swell properly on cooking) are better penetrated by the digestive ferments than in the latter where the grains cook to a pasty consistency [Sreenivasan, 1939].

Experiments with single enzymes acting on specific substrates are not by themselves quite sufficient. The human system secretes a variety of enzymes, all of which act on the highly complicated and mixed type of diets that are consumed. Thus, although the fat of rice bran has an inhibitory action on amylases, it may not do so in the human system which also secretes a lipase capable of hydrolysing the bran fat. In a similar manner, the inhibitory action of proteins will be eliminated through the action of pepsin and trypsin. There is great need therefore to work with mixtures of enzymes to obtain a correct indication of the digestibility of whole grains. Further researches along these lines are in progress.

The manner in which the present study on the digestibility of rice varieties has been carried out and the conditions of the digestions are such that the results obtained may not have any direct physiological application ; there is no doubt, however, that this study has yielded results of value on the comparative digestibilities of coarse and fine varieties of rice and on the effects of polishing and parboiling on such digestibilities. Further trials on animals or human beings are necessary to confirm these findings.

Summary

1. The digestibility of rice, raw or parboiled, by (pancreatic and salivary) amylases increases with the degree of polishing. Parboiled rice, especially when unpolished, is more easily digested than raw rice.

2. With a very high degree of polishing, there is a slight diminution in the extent of hydrolysis of rice starch. The viscosity of cooked rice flour increases with increased polishing, the rate of such increase being greater with a high degree of polishing.

3. With cooked whole grains of rice, parboiled rice starch is hydrolysed less easily than raw rice starch by pancreatic amylase. The rate of hydrolysis is determined by the extent of penetration of the digestive ferments into the grain.

4. Study of the digestibility of a large number of rice varieties has shown that invariably polished rice is digested more easily than unpolished rice and that parboiling somewhat increases the rate of digestion. There is, however, a large amount of individual variation between the different varieties.

5. Rice bran has a definite inhibitory action on amylases; the extent of such inhibition is greatest with the first layer of polishings and decreases steadily with the subsequent layers. Among the individual bran constituents the ether-extractives and the proteins of the bran exercise definite, though small, inhibitory action on starch hydrolysis by amylases.

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IV. STORAGE CHANGES IN RICE AFTER HARVEST

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I. Introduction

It is well known that freshly harvested rice is not fit for consumption and should be stored for some time before usage. Experience has shown that stored rice cooks better, yields less gruel and digests more easily than the unstored product. Old rice also sells at a higher price and is, besides, more popular.

The problem of storage is of general interest for, most grain and tuber crops need a preliminary period of storage before they can be safely consumed. Thus, wheat acquires 'strength' on storage and is unsuitable for milling and bread-making before being 'conditioned'. *Ragi* is valued more as a food when stored for some time during which period it is said to improve considerably. Fresh potato, in spite of its agreeable flavour and pleasant taste, causes digestive disorders due, it is believed, to high concentrations of certain salts and should therefore be kept aside for some time after harvest.

Although the need for storage in rice is generally well recognised, yet very little scientific information is available on the precise nature of the changes undergone by the starch and other constituents of the grain during storage. Freshly harvested rice is said to contain a toxic substance of an alkaloidal nature [Hofmeister, 1920] which disappears during storage. It has also been suggested that the absorption of water and consequent swelling takes place through certain small capillaries in the grain and that these capillaries widen during storage, thus allowing of greater imbibition of water [Subbaramiah and Rao, 1937]. According to another explanation, the starch of the grain is assumed to become progressively more complex during storage by a process of slow dehydration [Rao, 1935]. The above conclusions, however, have been drawn only from indirect evidence. In a recent communication, Sahasrabuddhe and Kibe [1935] have proved the existence, in the resting seed, of an active amylohydrolytic enzyme which has the power of attacking both gelatinized and ungelatinized starch of the rice grain and is, to a certain extent, responsible for the greater digestibility of stored rice compared to that of the new rice.

In the present paper, fresh evidence has been presented to show that (*a*) new, unstored rice does not digest as easily as stored rice, mainly because it cooks to a pasty condition and is not therefore penetrated by the digestive ferments as well as the properly swollen grains of the stored rice, and (*b*)

one at least of the factors responsible for the unstored rice cooking to a squashy consistency is the presence in the grain, immediately after harvest, of a fairly active α-amylase which, during storage, passes into a dormant condition [Giri and Sreenivasan, 1937].

II. Cooking quality of unstored and stored rices

The most conspicuous feature of well-stored rice is its capacity for swelling on cooking with water. New rice imbibes very little water and cooks to a paste. On storing, the rice develops a greater capacity for imbibing water. The extent to which this quality improves on storage depends on the method of storage and also the period for which the rice has been stored. In the following experiments, three different varieties of rice were stored as paddy immediately after harvest (1) in loose gunny bags, (2) in bottles loosely plugged with cotton wool and kept (*a*) at room temperature and (*b*) at 0°C. in the ice chest, (3) in tightly stoppered bottles and (4) in sealed flasks (*a*) anaerobically, and (*b*) in presence of carbon dioxide gas. The cooking qualities of the different samples were studied from time to time by measuring the volume expansion during cooking. This was done by displacement of a known volume of water with a definite quantity (usually 10 grms.) of the shelled whole rice grains both before and after cooking under standard and comparable conditions. The measurements were made in a specially improvised dilatometer.

An essentially similar device was employed by the Paddy Specialist at Coimbatore in his studies on the physical analysis and cooking qualities of market samples of rice (*Final Report of Work done at Coimbatore in connection with the Investigations on the Quality of Rice*, 1938, p. 12).

Cooking quality is given by the ratio of the volume of cooked grains to that of the uncooked grains. The results thus obtained are given in Table I.

Table I

Changes in cooking quality on storage

Storage treatment	Variety—Adt 11						Variety—Co 9						Variety—Co 10					
	Period of storage in months																	
	0	1/2	1	2	3	4	0	1/2	1	2	3	4	0	1/2	1	2	3	4
1	1·9	2·2	2·6	3·0	3·6	3·6	1·7	1·9	2·3	2·9	3·6	3·8	1·7	2·1	2·5	3·2	3·4	3·8
2 (*a*)	...	2·1	2·5	3·1	3·5	3·8	...	2·1	2·4	2·9	3·7	3·9	...	2·2	2·6	3·2	3·6	3·8
2 (*b*)	...	1·8	1·9	2·1	2·3	2·7	...	1·6	1·7	1·7	1·9	2·3	...	1·8	1·8	2·0	2·2	2·0
3	...	2·0	2·8	3·3	3·7	4·0	...	1·9	2·5	3·2	3·4	3·7	...	2·1	2·7	3·0	3·6	3·9
4 (*a*)	...	1·9	2·9	3·4	3·8	4·2	...	1·9	2·6	3·4	3·6	3·8	...	2·2	2·8	3·4	3·9	4·1
4 (*b*)	...	2·2	3·0	3·6	4·0	4·3	...	2·0	2·8	3·5	3·9	4·0	...	2·4	2·8	3·5	4·0	4·0

The results show that the cooking quality improves in samples stored in air-tight containers or under anaerobic conditions. Paddy stored in cold chamber does not improve in cooking quality even at the end of four months. In fact, some of the samples taken out after nearly eight months of storage were found to have scarcely improved in their swelling capacities on cooking. Well-stored rice grain swells on cooking to about four times the original volume while the unstored grain does not swell even to twice the original volume. The unstored grain also cooks more rapidly than the stored product. The foregoing observations were confirmed generally by the independent findings made simultaneously by the Paddy Specialist at the Rice Research Station, Coimbatore [K. Ramiah, *private communication*].

It has been observed at Coimbatore that when freshly harvested rice is stored in metallic bins with and without provision for ventilation, the improvement in cooking quality as a result of storage is slighly better in samples stored in ventilated bins than in those kept in closed containers; but this difference is observed only during the early stages and tends to fall off with the period of storage (*Final Report of Work done at Coimbatore*, 1938, p. 2).

It is not clear whether the storage changes have any bearing on the respiratory changes of the grain. The paddy of course continues to live and respire during storage. But, since storage changes are most rapid in properly closed pits or cisterns, it would follow that respiration is not entirely responsible for the attendant transformations.

Measurement of swelling on cooking is an elegant method of determining the extent of storage changes in rice. Another method will be to determine the extent of disintegration as a result of cooking from turbidity measurements or from the amounts of dissolved material. The latter is conveniently determined by precipitation with alcohol, filtering and weighing after drying.

With a view to finding out the extent to which polishing affects the swelling capacity of unstored and stored rices, some trials were carried out with freshly harvested rice and with rice stored for one year, both polished to different known extents [Sreenivasan *et al.*, 1938]. The cooking qualities, measured as before, are given in Table II.

TABLE II

Degree of polishing in relation to cooking quality of unstored and stored rices

Variety	Degree of polishing per cent				
	0	5	8	12	15
Adt 11, new . . .	1·9	2·2	2·4	2·9	2·9
Do. 1 year old . .	4·2	4·4	4·3	4·5	3·8
Co 9, new	1·7	1·9	2·4	2·9	3·2
Do. 1 year old . .	4·0	4·2	4·2	4·4	3·6

It is interesting to note that in the well-stored specimens, the cooking quality increases slightly with polishing until, with a very high degree of polishing, there is a definite decrease. The rice cooks in these cases rapidly and often to a shapeless consistency. With the unstored rice however there is a steady increase in swelling capacity with the extent of polishing. This observation is of especial significance and is again referred to in a later section.

III. Digestibility of cooked unstored and stored rices

It has been shown elsewhere [Sreenivasan and Giri, 1938] that when rice is cooked to a lumpy condition and then subjected to the action of amylohydrolytic enzymes, it does not digest even half as much as when cooked to the usual consistency. This difference in starch hydrolysis is exhibited even by stored rice when the latter is cooked to a paste (by overboiling with excess of water) and to a loose structure. Thus, in the following experiments, a specimen of well-stored (unpolished) rice was cooked in two lots (10 grms. each). In one case, cooking was done to the usual fluffy consistency while in another, the grains were allowed to cook to a squashy condition. The cooked grains were, in either case, added to a mixture of 150 c.c. of water and 50 c.c. of $M/15$ phosphate buffer at pH 6·8. The mixtures were kept in a thermostat at 35°±0·1° and, after some time, treated with 2 c.c. of a 0·5 per cent solution of trypsin (Pfansteil). Aliquots of the reaction mixtures were removed immediately after addition of the enzyme and at intervals of 10, 20 and 30 minutes and the reducing sugars estimated by the method of Willstätter and Schudel [1918]. The results, expressed in terms of mg. of maltose in the total volume of the reaction mixture, are given in Table III.

Table III

Effect of cooking to a paste on the digestibility of rice grains

Condition of cooked rice	Mg. maltose formed at the end of		
	10 mins.	20 mins.	30 mins.
Cooked to normal consistency	94	128	218
Cooked to a paste	55	94	122

The properly swollen grains present a greater reactive surface and are hence better penetrated by the digestive ferments than those which are not. A similar phenomenon has been observed by Reddi and Subrahmanyan [1937] who have studied the action of certain moulds on rice cooked to different extents. When the individual grains stand apart, the mould threads

penetrate them easily and convert them into sugar and other products whereas, when the rice is otherwise rendered squashy, the moulds penetrate only partially leaving the hard central portions unattacked.

The above observations were confirmed by some preliminary growth experiments on young rats. Two groups of four rats each were fed, for a period of thirty days, on cooked powdered rice and cooked whole rice respectively from the same variety. The rice was supplemented with small identical quantities of casein, cod-liver oil and salt mixture in both the cases. Weekly average increases in weight are given in Table IV.

TABLE IV

Growth rate with cooked powdered rice and cooked whole rice

Diet	Weeks			
	1	2	3	4
	Average growth rate in grms.			
Cooked whole rice	5·1	4·9	4·4	4·6
Cooked powdered rice . . .	5·1	4·6	4·0	4·3

The growth rate is poorer when the powdered grain is used. This naturally cooks to a paste compared with the whole grain. It would therefore appear that the bad effects of unstored rice are due primarily to its cooking to a shapeless pasty condition and thereby preventing the digestive ferments of the human system from coming into proper contact with the cooked mass. Part of the starch may pass undigested into the lower intestines and thus produce undesirable fermentations and other digestive disorders.

IV. *In vitro* DIGESTIBILITY OF THE STARCHES FROM NEW AND OLD RICES

Pure starches from new and one year old samples of rice from varieties Co 9, Adt 11, GEB 24, and Co 10 [Sadasivan and Sreenivasan, 1938] were prepared as follows :—

The shelled grains were soaked in 20 per cent alcohol and the softened kernels were then ground in a mortar. Enzymic action during the extraction was checked by the addition of $1 \times 10^{-4}N$ iodine [Olsson, 1921]. The pulps thus obtained were extracted thrice with cold, 20 per cent alcohol. The extracts were strained through several thicknesses of cloth and the starch removed by centrifuging and filtering. It was purified by washing in 20 and 80 per cent alcohol as described by Parker [1935]. The starch was washed and centrifuged in 80 per cent alcohol over ten times before it was considered pure. The air-dried specimens were powdered to pass the 100-mesh sieve.

For experiments on the digestibility of the starches by different enzymes, requisite quantities of the starches were treated with a little water, boiled for about five minutes and then autoclaved for thirty minutes at 15 lbs. pressure to ensure that the samples were gelatinized completely and under comparable conditions. Human saliva (1 : 1) and a 0·5 per cent solution of trypsin (Pfansteil) were employed for the studies. The reaction mixtures consisted of 20 c.c. of phosphate buffer (*p*H 6·8), 50 c.c. of one per cent starch suspension, 1 c.c. of normal sodium chloride and 27 c.c. of water. The mixtures were placed in a thermostat at 35°± 0·1° and, when the contents had acquired the temperature of the bath, 2 c.c. of the enzyme solution was added, thus making a total volume of 100 c.c. Aliquots (20 c.c.) of the reaction mixture were removed immediately after addition of the enzyme and at intervals of 5, 10, and 15 minutes and the reducing sugars present estimated by Willstätter and Schudel's [1918] method. The results are recorded in Table V as mg. of maltose formed in the total volume of the reaction mixture.

TABLE V

Digestibility of starch from new and stored rice

Starch from	Salivary Amylase			Pancreatic Amylase		
	Mg. maltose formed at the end of					
	5 mins.	10 mins.	15 mins.	5 mins.	10 mins.	15 mins.
Adt 11, new	159	186	207	122	186	245
Do. old	165	192	228	125	182	245
Co 9, new	196	254	286	141	206	275
Do. old	188	274	296	145	216	290
GEB 24, new	184	242	292	197	265	304
Do. old	184	246	302	194	272	312
Co 10, new	124	196	232	100	136	195
Do. old	118	196	226	122	146	226

It may be seen that though the pure starches from stored rice specimens digest more readily than those from the freshly harvested rices, the differences are not very pronounced. Sahasrabuddhe and Kibe [1935] have reported that the starches from new and old rices do not differ in their physical characteristics although they observe that under the action of hydrochloric acid,

diastase, and pancreatin, the starches from old rices are hydrolysed much quicker than those from new rices. It is possible that in the above experiments the mode of preparation of the starches has obliterated to some extent the differences, if any, in the make up of the starches of new and stored rices. Further work is essential to prove whether this is so or not.

V. Carbohydrate changes during ripening and storage

In view of the fact that carbohydrates predominate in rice, it was considered probable that storage may result in a change in these constituents more than in any other. Accordingly, specimens of rice (finely ground and passed through 60-mesh sieve) from freshly harvested and one year old samples of four different varieties were analysed for their carbohydrate constituents—dextrose, sucrose, other dextrins and polysaccharides, and starch —according to the methods of the A. O. A. C. [1935]. The results are given in Table VI.

Table VI

Carbohydrate constituents in new and stored rices

Variety	Moisture	Dextrose	Sucrose	Dextrins	Starch
	Percentages				
Adt 11, new . . .	13·2	2·12	0·36	1·86	74·8
Do. old . . .	11·8	2·02	0·36	1·34	74·5
Co 9, new	13·4	2·65	0·48	1·56	74·6
Do. old . . .	12·0	2·32	0·43	1·11	74·6
Adt 6, new . . .	11·9	1·85	0·30	1·80	72·2
Do. old . . .	10·4	1·79	0·29	1·26	72·0
Co 10, new	12·6	1·45	0·34	2·05	74·9
Do. old . . .	12·1	1·38	0·36	1·85	73·7

There does not appear to be any marked change in the different carbohydrate constituents as a result of storage.

Storage changes are often considered to be mere continuation of the changes during ripening. To verify this, the changes in carbohydrate constituents were followed from the time the grain began to set. Samples (variety Adt 11) in 2 lb. lots were sent to Bangalore from the Agricultural Research Station, Coimbatore, by Railway Parcel. They were taken from the crop

in the field every fourth day. The time taken for transit varied between two and four days and hence a certain amount of change must have taken place during this period. With the available facilities, this could not be checked. The first sample was taken after the grain had begun to set. In all, nine samples were collected, the last sample representing the fully ripe grain at the harvesting stage. Each sample of paddy was analysed as before for the different carbohydrate constituents immediately after receipt. The harvested sample was also examined at fortnightly intervals during storage. Table VII gives the results obtained.

TABLE VII

Carbohydrate changes in paddy during ripening and storage

Stage of ripening	Moisture	Reducing sugars	Non-reducing sugars	Soluble starch, dextrin, etc.	Starch *
	Percentages				
First	8·7	7·96	0·54	7·42	39·21
Second	8·8	7·01	0·33	6·68	55·42
Third	8·9	7·02	0·38	6·64	58·89
Fourth	8·5	6·45	0·47	5·98	59·95
Fifth	8·7	5·00	0·37	4·63	61·86
Sixth	8·2	4·57	0·25	4·32	62·12
Seventh	8·6	4·59	0·31	4·28	62·19
Eighth	7·1	3·07	0·38	2·69	62·96
Harvest sample	8·1	2·74	0·41	2·33	64·00
Stored—					
(1st fortnight)	8·5	2·68	0·38	2·30	64·15
(2nd fortnight)	8·4	2·71	0·39	2·32	64·26
(3rd fortnight)	8·2	2·72	0·40	2·32	63·85
(6th fortnight)	8·2	2·59	0·35	2·23	65·02

* Determined by taka-diastase hydrolysis followed by Widdowson's [1931] method of sugar estimation.

It may be observed that during ripening there is a steady decrease in reducing sugars, sucrose and dextrin and a rapid increase in starch content. There is however no marked change in these constituents during storage (Table VI).

VI. THE AMYLASE SYSTEM OF THE RICE GRAIN DURING RIPENING AND STORAGE

Rice grains (variety Co 10) were freshly collected from the crop at different stages of ripening. The fresh weights and the moisture contents were determined immediately. The grains were extracted with 13·5 times their

weight of water for twenty-four hours at room temperature. The amount of extractant to be added was always calculated on the dry weights of the grains taken.

The saccharifying power (β-amylase) and the dextrinizing power (α-amylase) of the extracts were determined according to methods outlined by Giri and Sreenivasan [1937, 1938] at *p*H 4·6 (*M*/5 acetic acid-acetate buffer) and 7·0 (*M*/15 phosphate buffer) respectively, these being the *p*H optima of the two amylases [Giri and Sreenivasan, 1936]. The results are presented in Table VIII.

TABLE VIII

Variation in the activities of α- and β- amylases of rice grain during ripening and storage

Stage of grain	Weight of 200 grains grm.	Per cent moisture	β-amylase (at *p*H4·6)		α-amylase (at *p*H7·0)	
			Time of hydrolysis in hours	Activity in mg. maltose in total volume of reaction mixture	Time required in mins. to change colour tone of iodine to	
					Violet	Rose
'Milk' stage .	3·937	46·2	1 3 24	8·6 47·0 202·0	30	1200
'Mealy' stage	4·710	37·0	1 3 24	8·6 47·0 200·0	30	1200
Ripe grain .	5·992	24·5	1 3 24	8·3 26·0 90·0	1400	..
Stored for one month.	4·740	16·5	1 3 24	7·5 23·0 77·0	1400	..

It will be seen that the activities of both the amylases decrease as ripening advances, while in the resting grain it is almost completely *nil*. It has been shown elsewhere [Giri and Sreenivasan, 1937, 1938] that the amylases in the resting grain are present in a dormant condition and that they can be obtained in a fully active state by extraction with phosphate buffer at *p*H 7·0.

In the following experiments, the activities of the two amylases have been followed for two and a half months during storage, in three varieties of paddy from the time of harvest (Tables IX and X).

TABLE IX

Changes in β-amylase activity during storage

Variety *	Date of harvest	Time of hydrolysis in hrs.	Date of sampling: 24-6-37	6-7-37	21-7-37	18-8-37	1-9-37
			Activity in mg. maltose in total volume of reaction mixture				
Halublu (I)	12th June 1937	1	11·4	9·2	6·8	4·8	4·3
		3	39·2	18·6	9·8	11·9	8·9
		24	92·6	74·2	55·0	44·6	33·8
Kempasanna (II)	8th June 1937	1	10·8	7·6	4·4	8·6	*Nil*
		3	38·6	23·9	14·6	12·2	8·8
		24	74·2	94·6	74·6	65·7	33·6
Three month crop (III)	16th June 1937	1	10·2	4·8	*Nil.*	4·3	*Nil*
		3	29·6	16·2	8·5	9·8	4·8
		24	77·0	68·4	42·4	38·6	26·0

* These varieties were obtained from the Hebbal Agricultural Farm through the courtesy of the Superintendent.

TABLE X

Changes in α-amylase activity during storage

Time in mins.	Date of sampling: 24-6-1937			6-7-1937			21-7-1937			18-8-1937			1-9-1937		
Variety	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
	Colour tone with *N*/200 iodine solution														
15	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
30	BV	BV	B	..	..	..	..	..	..	..	..	..	..	..	..
45	BV	BV	BV	BV	B	B	BV	B	B	..	..	..	..	..	..
60	..	..	..	BV	BV	BV	BV	BV	B	BV	B	B	..	..	..
90	..	..	..	..	..	..	BV	BV	BV	..	..	..	BV	B	B
120	V	BV	BV	..	..	..	..	..	..	BV	BV	B	BV	B	B

B = Blue; BV = Blue-violet; V = Violet.

The changes in cooking quality as measured by the extent of volume increase during cooking for the three varieties and at the corresponding dates of sampling are set forth in Table XI.

TABLE XI

Changes in cooking quality during storage

Variety	Date of sampling				
	June 24th	July 6th	July 21st	Aug. 18th	Sept. 1st
Halublu	2·4	2·9	3·3	3·4	3·4
Kempasanna . . .	2·6	3·2	3·6	3·9	4·1
Three month crop . .	2·0	3·0	3·9	4·1	4·2

It may be seen that as before (with Co 10, *vide* Table VIII) the activities of the amylases decrease with the period of storage. There is a close similarity observed between the α-amylase activity and the extent of swelling during cooking. Thus with the Halublu variety, the dextrinizing enzyme does not become as rapidly inactive with storage as with the other two varieties. Likewise, the improvement in cooking quality is less pronounced in this than in the other two varieties.

VII. DISCUSSION AND GENERAL CONCLUSIONS

The symptoms attending the consumption of unstored rice are essentially those of poor digestion. One feels heavy shortly after eating such rice and sometimes complains of stomach pains and occasionally even of diarrhoea. These only suggest that unstored rice is slow to digest; that a part of it passes into the smaller intestines and colon, and, being acted there by the putrefactive bacteria, causes fermentation followed by the usual symptoms of diarrhoea.

It has been shown above that although new rice is really more cooked than the stored grain, the digestive juices do, nevertheless, find it difficult to attack it with the same facility as the latter. Cooked, unstored rice being lumpy and held together by the adhering gruel, presents less reactive surface to the action of the ferments.

A further observation which is of some practical significance in connection with the ill-effects of unstored rice is that it is generally consumed in larger quantities. This is due to the fact that it occupies much less volume than cooked stored rice so that a proportionately larger quantity is eaten before the consumer gets the desired sense of satisfaction.

This raises the question as to why unstored rice cooks always to a paste. It is interesting to note that there is present in the grain, immediately after harvest, an enzyme capable of liquefying or dextrinizing the starch and that this enzyme becomes dormant and inactive when the grain is stored for some time. This α-amylase may be responsible for fresh rice cooking to a squashy condition. The enzyme is very resistant to heat and is not inactivated except on heating to a temperature of about 55° to 60°C. [Yamagishi, 1937]. Rice is usually cooked by adding the grains to water kept nearly boiling. This process of cooking takes a certain time and the grains do not attain the temperature of boiling water until after some time. During this period of cooking, there is obviously, considerable action by the α-amylase on the starch resulting in partial liquefaction.

That it is probably so has been tested in a number of ways. Thus, the pastiness of fresh rice on cooking was found to be greater when this rice was added initially to water and the mixture brought to boil. In this case, the enzyme acts on the starch evidently for a greater length of time before getting inactivated. Again, when fresh rice was dry-heated at about 60° for two or three hours and then cooked, its swelling capacity was greatly improved and compared well with that of the well-stored product. This is due no doubt to the inactivation of the α-amylase during the drying. Mere storage at ordinary temperatures for extended periods also results in such inactivation, but, whereas here the enzyme only passes into a dormant condition [Giri and Sreenivasan, 1937], it is completely destroyed in the former case.

Study of the distribution of the liquefying and saccharifying components of the amylase system in the different parts of the rice grain has shown that these—especially, the liquefying amylase—are mostly localised in the peripheral layers and the germ [Giri and Sreenivasan, 1938 ; Yamagishi, 1937]. These are the very fractions that are lost during the polishing of rice. Hence it is—probably—that the swelling capactity on cooking of unstored rice increases with polishing (Table II).

It is well known that parboiling is one of the potent methods by which freshly harvested rice can be made to swell to its full and proper consistency and thus rendered fit for consumption. This would suggest that the process of heating during parboiling destroys the liquefying amylase. Parboiled rice has a translucent appearance because of the fact that there is partial dextrinization of the starch during parboiling [Sreenivasan *et al.*, 1938]. This might itself have been brought about by the enzyme before destruction.

Quite apart from the inactivation of the starch-splitting ferment, there may be other factors also responsible for the storage changes in rice, which are not properly understood.

A great deal of useful information has, however, been gathered with regard to the methods of storage and means of hastening the same. Thus, as already mentioned, the storage changes take place most rapidly in closed

containers well insulated with adequate plastering against loss of heat by radiation. Increased temperature, up to a point, favours storage changes. On the other hand, a lowered temperature causes distinct retardation.

Short-duration varieties need longer periods of storage than long-duration ones. A few varieties, like GEB 24, become fit for consumption almost immediately after harvest [K. Ramiah, *private communication*]. Paddy harvested in the warm and dry months requires considerably lesser time for storage than that obtained during cold and wet weather. Harvesting practices essentially affect the extent of storage changes necessary and, hence, the time of storage is often a relative feature not entirely dependent upon the variety involved [Srinivasan, 1934].

Although storage by itself involves very little expense, yet neither the producer nor the consumer will like to have the rice on hand for such a long period as it now requires. Quickening of the related changes is highly desirable and it will therefore be of some practical interest to devise improved methods of storage.

Drying in a current of air at 50° to 60° for a few hours is indeed an efficient method of hastening storage changes, but the great drawback in practice of such a method is the development of 'sun-cracks' in the grain, due to partial dehydration at various points in the surface. The cracks disappear during cooking but they do, nevertheless, affect the shelling and milling qualities of the grain adversely.

The observation, that the difference in digestibility between unstored and stored rice is not exhibited to the same degree by the pure starches prepared out of them (Table V), would suggest that there is probably not much difference in the make-up of the starch in the two cases. Further work is essential to elucidate the precise nature of the starch and other constituents in rice and their changes during storage. In some experiments, the problem of storage in rice was studied in relation to (*a*) the limit of saccharification of the starch in the grain, and (*b*) the extent of fractional liquefaction of starch at different temperatures [Warth and Darabsett, 1914]. The bearing of these results [Sreenivasan and Giri, *unpublished data*] on the quality of unstored and stored rices, as also the changes in the starch components of the grain during storage, are being investigated and will be reported in a future communication.

Study of the degradation of starch by different amylases would yield results that may throw light on certain features of its constitution that have not hitherto been approachable by purely chemical means [Hanes, 1937]. A further line of investigation would consist in measuring quantitatively the change of colour with iodine and establishing the relationship between this and the stage and type of degradation that has been induced.

VIII. Summary

1. Well-stored rice grains swell on cooking to about four times the original volume while the unstored grains from freshly harvested paddy scarcely swell to twice the original volume.

2. Increased temperature and reduced air supply during storage quickly improve cooking quality of unstored rice. Paddy stored in cold chamber does not improve much in cooking quality even after several months.

3. Polished, unstored rice swells on cooking to a greater extent than the corresponding unpolished specimen; polishing also improves cooking quality of stored rice, though with a high degree of polishing swelling capacity decreases.

4. *In vitro* experiments have shown that when rice is cooked to a paste, it does not digest even half as much as when cooked to the usual fluffy consistency. The properly swollen grains present a greater reactive surface and are better penetrated by the digestive ferments than those which are not. Growth rate in young rats fed on cooked powdered rice is also less compared with that on rats fed on cooked whole rice.

5. There is only a small though definite difference in the *in vitro* digestibilities of the pure starches prepared out of freshly harvested and well-stored specimens of rice. The latter is hydrolysed more easily than the former.

6. During the ripening of the rice grain, there is a steady decrease in the reducing and non-reducing sugars and dextrin followed by a rapid increase in the starch content. During storage, there is no marked change in the amounts of the various carbohydrate constituents of the grain.

7. The resting seed contains both a liquefying (α) and a saccharifying (β) amylase. The activity of the amylases is greatest at the 'milk stage' of formation of the grain and decreases with the advance in ripening; there is a further decrease in their activity during the storage of the rice grain. In the well-stored grain, the amylases exist in a dormant condition and cannot be extracted with water. There is a close parallelism between the decreased activity of the α-amylase during storage and the improvement in swelling capacity on cooking.

8. The bearing of these observations on the mechanism of storage changes in rice and the nature of further work is discussed. It is concluded that the pastiness on cooking, of unstored rice may, to a certain extent, be due to the starch liquefying ferment (α-amylase) of the seed; the inactivation of this enzyme during storage is followed by increased swelling capacity on cooking.

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ENTOMOLOGICAL INVESTIGATIONS ON THE LEAF-CURL DISEASE OF TOBACCO IN NORTHERN INDIA

III. THE TRANSMISSION OF LEAF-CURL BY WHITE-FLY, *BEMISIA GOSSYPIPERDA*, TO TOBACCO, SANN-HEMP AND A NEW ALTERNATE HOST OF THE LEAF-CURL VIRUS

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(With Plates VII-X and one text-figure)

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I. Introduction

In the second part of our investigations on tobacco leaf-curl [Pruthi and Samuel, 1937] we stated that sann-hemp at Pusa also suffers from leaf-curl and described some transmission experiments performed during 1935 which showed that white-fly, *Bemisia gossypiperda* M. & L., could transmit the disease from sann-hemp to healthy tobacco. During the last two tobacco seasons (1936-37 and 1937-38) we have performed a large number of transmission experiments with white-fly to confirm our previous conclusions referred to above and to ascertain if this insect could also transmit the disease from tobacco back to healthy sann-hemp and from tobacco to tobacco and sann-hemp to sann-hemp. Furthermore, during the course of our work we have observed that *Ageratum conyzoides*, which is a very common weed at Pusa and which also suffers from a leaf-curl disease, serves as a food plant for the white-fly. We have experimented with this weed to determine if this plant, like sann-hemp, is an alternate host of the tobacco leaf-curl virus. Several large series of experiments on the lines indicated above have been performed at different times of the year on tobacco and other hosts of different ages to determine the season when the white-fly is most viruliferous and the ages of various hosts when they most readily get infected and develop the disease. Some very interesting results have been obtained which are reported in the present paper.

II. Technique, material, etc.

In our previous investigations [Pruthi and Samuel, 1937] we used cellophane tubes for encasing the white-fly on the diseased and healthy plants for feeding. As whole leaves had to be enclosed inside the tubes, the latter had necessarily to be very large. It was noticed that the white-flies generally remained in the corners and folds of the crumpled portions of the tubes instead of sitting and feeding on the enclosed leaves. As the longevity of the white-fly in captivity is rather short, it seemed probable that the encased individuals did not feed on the diseased plants at all or, if they did so, they did not feed for sufficient time on the healthy plants on which they were transferred. Furthermore, cellophane has the very serious draw-back in that with slight changes in weather conditions it readily expands and shrinks and consequently becomes very brittle. Therefore in the experiments carried out during the last two years and reported in the following pages, the technique of feeding the white-fly was considerably modified. Instead of a large cellophane tube for encasing an entire leaf, a small glass tube (1 in. $\times \frac{1}{4}$ in.) containing the individuals was fixed on a portion of the leaf by means of a light wire spring which clasped the tube and leaf together as shown in Fig. 1. To avoid injury to the leaf, the end of the spring which rested on it was flattened into a disc and was separated from the leaf surface by means of a felt piece. The white-fly generally feeds on the lower surface of the leaf, therefore, the tubes were also fixed on that surface. This as well as the fact that the white-fly always flies up when put in a tube ensured the settling of the individuals on the portion of the leaf covering the open end of the tube.

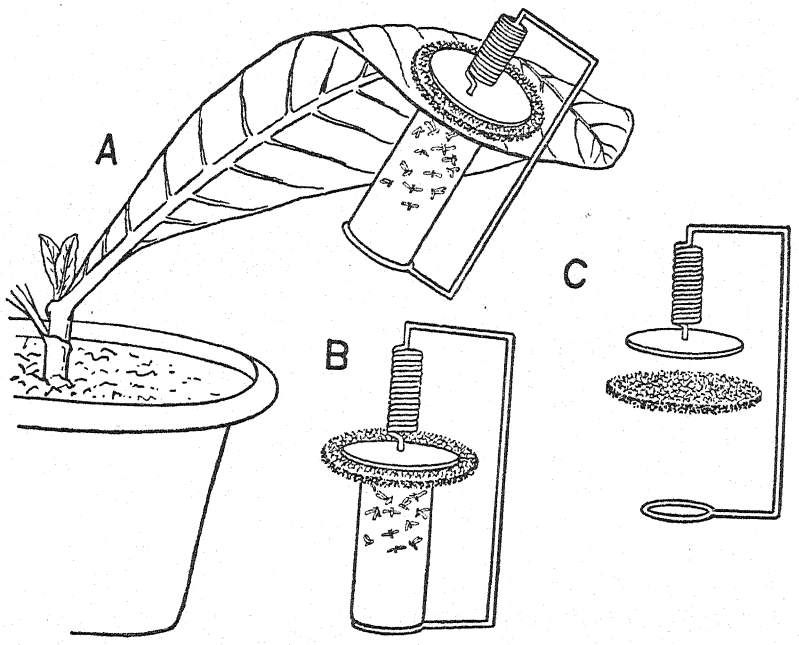


FIG. 1. Diagrammatic illustration of the technique of feeding white-flies on tobacco leaf—*A*. The whole mechanism *in situ*; *B*. The spring and the tube containing the individuals; *C*. The spring showing the felt piece separated from the disc.

The period for which the white-flies were kept on the diseased plants before their transfer to healthy hosts is referred to as the 'feeding period' in the following pages. This, however, is not a very accurate term as the individuals did not necessarily feed for all the time when encased on the leaves After the individuals had been kept on diseased leaves for a definite period. they were transferred to healthy plants on which they were allowed to remain till they died. This was done to ensure their feeding on these plants for the maximum possible period as the percentage infection is known to increase with increased feeding time of the vector on the healthy plant. No record was kept of the period for which the white-flies lived on healthy plants. The total longevity of the white-fly while encased on diseased and healthy, plants varied between three and seven days during August-December. When kept on diseased leaves throughout its life, it did not live for more than about four days. If kept on healthy plants throughout, it remained alive for six to ten days. These observations do not support the statement of Smith [1931] that an infective insect does not present any visible variation from a normal individual.

Tobacco seedlings meant for infection purposes and the controls were raised under insect-proof conditions and were transplanted in pots containing sterilized soil which were kept covered with muslin bags throughout the life

of the plants. Similarly sann-hemp and *Ageratum* plants were kept under insect-proof conditions.

The work was carried out at Pusa (Bihar) and most of the transmission experiments were performed during August-December, the usual period of active growth of tobacco crop in Bihar, as it is recognized [Smith, 1935] that it is difficult to infect mature plants with virus diseases.

The infected plants were kept under observation up to the middle of the following April for noting the appearance of the disease. By this date all the tobacco plants both in pots and in the field had flowered and formed capsules.

The variety of tobacco under investigation was H 142, which is the most common cigarette tobacco grown at the Botanical Sub-station at Pusa.

The symptoms of the various types of leaf-curl of tobacco have not been described in the following pages, as Pal and Tandon [1937] have already published a detailed account thereof. We may, however, state that almost all the types undergo some change with the growth of the tobacco plant and towards the end of the season show some symptoms which have been described by Pal and Tandon as characteristic of *X* type. It may also be added that *A* type always starts as *X* type and in the course of two or three weeks acquires the typical characteristic of *A* type, therefore the two types cannot be distinguished from each other for sometime except that the plants which are going to develop *A* type have more severe symptoms of *X* type than those which are going to remain of *X* type. We have observed the same changes in the field and, therefore, venture to suggest that the descriptions of various types given by Pal and Tandon need some modifications in reference to the changes which the types undergo during different seasons of the year.

III. Transmission of the disease from sann-hemp to tobacco

Five series of experiments were performed between September and the middle of December 1936 on tobacco seedlings, eight to sixteen weeks old. The diseased sann-hemp used as source for these experiments had been sown in the field about the 1st July. The detailed particulars of the experiments are given in Table I-A.

In the first series, seven experiments were performed between the 6th and 12th September, when sann-hemp and tobacco under experiment were both about eight weeks old. Two to five specimens of the white-fly were kept on a diseased sann-hemp plant for twenty-four to forty-eight hours before they were transferred to a healthy tobacco seedling. None of the seven infected seedlings developed the disease.

In the second series, eight experiments were performed between the 20th and 29th September with both sann-hemp and tobacco ten to twelve weeks old. Two to twelve white-flies were kept on diseased sann-hemp for eighteen to fifty-three hours before they were transferred to a healthy tobacco seedling. After about thirty-six days one seedling (No. 1) out of eight developed leaf-curl of *A* type.

The third series consisted of ten experiments performed in the beginning of November with tobacco and diseased sann-hemp being seven and seventeen weeks old respectively. Nine to twenty-six individuals were used and they were kept for 18½ hours on the diseased source. Five out of ten infected tobacco seedlings (Nos. 1 and 7—10) developed leaf-curl. Three seedlings developed leaf curl of *A* type, and in the other two the disease was of *X* type. Whereas in the seedlings which developed *A* type, the disease was evident in about seventeen to nineteen days only, in those which developed *X* type it took as many as seventy-four and eighty-four days to appear.

At about the same time (beginning of November), seven more experiments were performed (series IV) on sann-hemp and tobacco, both seventeen weeks old. Seven to seventeen white-flies which had fed for twenty-four hours on diseased sann-hemp were put on a healthy tobacco seedling. None of the seven infected plants developed leaf-curl.

Again about the middle of December when the diseased sann-hemp was about twenty-two weeks old, ten experiments were performed on about eight weeks old tobacco seedlings (series V). Two seedlings developed leaf-curl in thirty-seven and sixty-three days respectively. The disease was of *X* type.

All the controls in the five series of experiments described above remained free from leaf-curl.

From the foregoing it will be evident that in 1936, as in the previous year, the white-fly was found capable of transmitting the leaf-curl virus from diseased sann-hemp to healthy tobacco which consequently developed disease of *A* type in most cases. It will be further noticed that the highest percentage of positive results (50 per cent) was obtained if the tobacco seedlings were about seven weeks old at the time of infection, as was the case in series III. Experiments performed at the same time and under the same conditions as those in series III but on seventeen weeks old tobacco gave much fewer positive results.

In the course of experiments carried out in 1936 and described above it was observed that with diseased sann-hemp as the source, some of the infected tobacco seedlings developed *A* type while others developed *X* type. In 1937 the symptoms of leaf-curl of sann-hemp were carefully studied before experiments were performed. Two distinct types of the disease could be made out. In one the leaf blades were simply curled but not puckered and there was only a slight thickening and greening of the veins. The second type was much more severe and was characterised by distinct puckering and twisting of leaf blades. In Plate VII, figs. 1 and 2 the two types are illustrated. They are designated as S.V.1 and S.V.2 in the following pages.

Five series of experiments were performed during 1937, two using S.V.1 type and three with S.V.2 type as source. The particulars of experiments

under various series are given in detail in Table I-B, and are briefly summarized in the following statement :—

Main results of transmission experiments from sann-hemp to tobacco, during 1937 (detailed particulars in Table I-B)

Series	Time of experiment	Age of tobacco infected (weeks).	No. of white-flies and the period for which they fed on diseased sann-hemp	Type of disease used as source	No. of plants infected	No. of plants which developed disease and their type	Remarks
			No. / hrs.				
I	9th-11th Sept. 1937	8	6—14 / 29	S.V.1	12	10, *X* ; 2, *D* ?	One *D* recovered
	Do. . .	8	7—14 / 24	S.V.1	28	2, *X* ; 2, *X* ?	Three recovered
II	27th Sept. to 1st Oct. 1937	7	9—27 / 20—48	S.V.1	27	22, *X* .	
III	27th-29th Sept. 1937	7	10—27 / 20—48	S.V.2	3	2, *A*	
IV	31st Oct. to 2nd Nov. 1937	7	5—25 / 24—29	S.V.2	27	15, *A*	
V	25th-28th Nov. 1937	6	3—7 / 24	S.V.2	25	1, *A*	

N. B.—None of the controls developed leaf-curl.

From the foregoing it will be evident that the white-fly can transmit both types of leaf-curl from sann-hemp to tobacco, S.V.1 and S.V.2 types giving rise to *X* and *A* types respectively in tobacco. Photographs of the tobacco plants in which *A* type was produced by experiment are reproduced in Plate VIII, figs. 1*a-h*. Figs. *j-k* are of the controls.

The least amount of time tried for which the white-fly was kept on the diseased source before it was capable of transmitting the disease was twenty hours. The maximum number of positive results was obtained from September up to the middle of November, if the tobacco seedlings at the time of infection

were six to eight weeks old. During the second half of November and December, even if the infected tobacco was of this age, the percentage of positive results was smaller.

Thus the results obtained in 1936 and 1937 clearly confirm those of 1935 in that sann-hemp is an important host of some types of tobacco leaf-curl and that the white-fly, *Bemisia gossypiperda*, can easily transmit the disease from sann-hemp to tobacco.

IV. Transmission of the disease from tobacco to sann-hemp

It has been shown above that the white-fly can easily transmit leaf-curl of both types (S.V.1 and S.V.2) from sann-hemp to tobacco. During 1936, four series of experiments were performed to ascertain if this insect could transmit the disease in the opposite direction, *viz.*, from tobacco to sann-hemp. The detailed particulars of the experiments are given in Table II-A. One to thirty white-flies were kept on diseased tobacco of *A* and other types for eight to forty-eight hours and were then transferred to healthy sann-hemp plants, nine to sixteen weeks old. Thirty-two plants were thus infected between the 12th of September and 6th of November. The sann-hemp seedlings under inoculation had been sown in the beginning of July. Only three infected plants (series III, No. 1-3) showed some reaction but none developed typical leaf-curl.

The incidence of leaf-curl in sann-hemp crops at Pusa is very high during the last week of July and whole of August. For the disease which appears at this time, the infection probably takes place some time before the middle of July. The failure to transmit the disease from tobacco to sann-hemp in the experiments referred to above seemed to be probably on account of the fact that sann-hemp at the time of experiments (September-November) had passed the growth stage at which it was susceptible to the infection. We could not perform any transmission experiments during July as no diseased tobacco for using as source was available before September. However, we kept by careful tending some diseased tobacco plants of 1936 for performing experiments on sann-hemp during July-August of the next year (1937), which are described below.

With the diseased tobacco plants of 1936 as source, four series of experiments were performed during 1937, full particulars of which are given in Table II-B, and the results are briefly summarized in the following statement :—

Main results of transmission experiments from tobacco to sann-hemp during 1937 (detailed particulars in Table II-B)

Series	Time of experiment	Age of sann-hemp infected (weeks)	No. of white-flies and the period for which they fed on diseased tobacco	Type of disease used as source	No. of plants infected	No. of plants which developed disease and their type	Remarks
			No. / hrs.				
I	16th-17th July 1937	$2\frac{1}{2}$	2—6 / 4—16	*X*	17	1, S.V.1	
II	18th-23rd July 1937	$2\frac{1}{2}$—3	3—7 / 8—13	*C*	5	1, S.V.1	
	Do. . .	,,	,,	*A*	13	1, S.V.2	
	Do. . .	,,	,,	*X*	4	2, S.V.1	
III	23rd-29th July 1937	3—4	1—9 / 6—18	*C*	8	3, S.V.1	
	Do. . .	,,	,,	*A*	10	..	
	Do. . .	,,	,,	*D*	3	..	
	Do. . .	,,	,,	X	4	..	
IV	27th-31st Aug. 1937	8	2—25 / 6—17	*C*	3	1, S.V.1	
	Do. . .	,,	,,	*C*+*D*	6	1, S.V.1	
	Do. . .	,,	,,	*A*	13	6, S.V.2	
	Do. . .	,,	,,	*X*	2	..	

From the above statement it will be clear that white-fly could transmit leaf-curl from tobacco back to sann-hemp, though the proportion of positive results (about 18 per cent) was much smaller than in the case of the transmission of the disease from sann-hemp to tobacco. The disease generally appeared three to five months after infection. A typical sann-hemp plant in which leaf-curl (S.V.2 type) was produced by experiment is illustrated in Plate VII, figs. 4 and 4*a*.

It will be observed that when diseased tobacco used as source was of *A* type, sann-hemp developed S.V.2 type and when it was *X* or *C* type, it developed S.V.1 type.

From the review of the results obtained in 1936 and 1937 it is evident that sann-hemp can get the infection from tobacco generally during July and August only, and that too in a small number of cases. As diseased tobacco is not in the field in North Bihar before the end of September or October, there is very little chance of this crop acting as a source of infection for sann-hemp.

V. Transmission of the disease from sann-hemp to sann-hemp

In 1936 two series of experiments were performed to ascertain if the white-fly could transmit the virus from diseased sann-hemp to healthy sann-hemp. The particulars of the experiment are given in detail in Table III-A. The first series consisted of twenty-four experiments in which four to twelve specimens of the white-fly kept on a diseased plant for twenty-four hours (between the 8th-13th October) were put on a healthy plant which was thirteen to fourteen weeks old at that time. Four out of twenty-four plants thus infected (Nos. 1, 7, 8 and 19) showed leaf-curl almost similar to that of the source. The disease took 57, 29, 27 and 136 days respectively to appear. In all the cases the side shoots of the plants also showed leaf-curl. The second series consisted of ten experiments in which eleven to twenty-seven white-flies which had been allowed to feed on diseased plants for nineteen hours on 4-5th November were introduced on about four months old healthy plants. One plant (No. 9) showed leaf-curl on 6th December. Its side shoots were also diseased.

Thus the white-fly could transmit leaf-curl from diseased to healthy sann-hemp, though the percentage of successful transmissions was rather low (10-16 per cent).

In 1937 two series of experiments were again performed on the above lines to verify the results obtained in the previous year. The particulars of the experiments are set forth in Table III-B. In the first series, consisting of eight experiments, type S.V.1 was used as source. Infections were done in the first week of September when the infected plants were about nine weeks old. Two to five white-flies were kept on a diseased plant for twenty hours before they were transferred to a healthy plant. Two out of eight infected plants developed leaf-curl after seventy-one and seventy-nine days respectively.

In the second series, consisting of nine experiments, diseased plants of type S.V.2 were used as source. Other conditions, *e.g.*, the number of white-flies used, the period for which they were kept on the diseased plants, the age of the infected plants, etc., were the same as in series I above. Two out of nine infected plants developed leaf-curl similar to that of the source after sixty-seven to seventy-two days.

Thus the results of two years' experiments show that white-fly can transmit leaf-curl of both types from sann-hemp to sann-hemp, but the percentage of positive transmissions (10-20 per cent) is small.

VI. Transmission of the disease from tobacco to tobacco

To ascertain the role played by the white-fly in the transmission of leaf-curl of various types from diseased to healthy tobacco plants, seven large series of experiments were performed at different times during 1936, the particulars of which are given in detail in Table IV-A and the results are summarized briefly in the following statement:—

Main results of the experiments performed for the transmission of leaf-curl from diseased to healthy tobacco during 1936 (detailed particulars in Table IV-A)

Series	Time of experiment	Age of tobacco infected. (weeks)	No. of white-flies and period for which they were fed on diseased plant	Type of disease used as source	No. of plants infected	No. of plants which developed the disease of various types	Remarks
1	2	3	4	5	6	7	8
			No. / hrs.				
i	14th-29th Sept. 1936	9—11	5 / 19	*B*	3	*Nil*	...
			3—12 / 16—48	*C*	8	1, *C*	
			1—3 / 16—17	*X*	6	1, *X*	
ii	8th-15th Oct. 1936	12—13	3—5 / 8—9	*A*	5	1, *X*	The source was not pure *A*.
			1—7 / 24	*B*	5	*Nil*	
			3—6 / 8—24	*C*	7	*Nil*	
iii	16th-17th Oct. 1936	9	2—5 / 7—8	*X*	4	2, *X*	
iv	28th Oct.-6th Nov. 1936	6—7	7—9 / 24	*A*	5	2, *C*; 1, *X*	The source was not pure *A*.
			3—13 / 5—24	*B*	16	5, *B*; 1, *AX*	
			4—12 / 5—24	*C*	24	3, *C*; 1, *X*; 2, *C*+*D*	
			4—7 / 24	*CX*	2	1, *CX*; 1, *CD*	
			26—33 / 21	*A*+*B*+*C*+*D*	4	2, *X*	The vectors were fed alternately on diseased plants of types *A*, *B*, *C* and *D* before being put on healthy plants.

Series	Time of experiment	Age of tobacco infected. (weeks)	No. of white-flies and period for which they were fed on diseased plant	Type of disease used as source	No. of plants infected	No. of plants which developed the disease of various types'	Remarks
1	2	3	4	5	6	7	8
			No. / hrs.				
v	14th-16th Dec. 1936	8	3—16 / 6—24	*A*	8	*Nil*	
			3—11 / 6—24	*B*	8	2, *B*	
			5—13 / 6—24	*C*	8	2, *C*	
			4—14 / 6—7	*D*	8	*Nil*	
vi	25th Dec. 1936 to 5th Jan. 1937.	6—8	4—7 / 18	*A*	10	2, *A* ?	
			2—5 / 24	*B*	8	1, *B* ?	
			3—9 / 18—24	*C*	18	1, *C*	
			4—9 / 6	*D*	10	1, *D* ?	
			2—8 / 24	*X*	8	1, *X*	
			11—24 / 6	*A*+*B*+*C*+*D*	7	3, *X*	The synthetic source as in Ser. *iv.*
			9—15 / 6	*A*+*C*	3	1, *X*	The synthetic source as in Ser. *iv.*
			7—11 / 6	*A*+*D*	3	2, *X*	The synthetic source as in Ser. *iv.*
vii	6th-9th Feb. 1937	7	4—11 / 18	*A*	15	1, *X*	
			4—12 / 18	*B*	15	4, *B*	
			5—13 / 18	*C*	15	*Nil*	
			6—11 / 18	*D*	20	3, *X*, 2, *D* ?	
			5—9 / 24	*X*	15	5 *X*	

From the statement given above it will be noticed that though on the whole the percentage of positive results obtained was rather low, white-fly could transmit all types of leaf-curl from diseased to healthy tobacco individually and synthetically. Furthermore, it is evident that the fly was most capable of transmitting the disease between the end of September and the middle of November and that tobacco was most susceptible to developing the disease if it was infected when about eight weeks old. Both the time of the year and the stage of growth of tobacco plants when the infections are made thereto have important influence on the development of the disease. The percentages of positive results from infections made during second half of September, October-November and December-January, were 12, 35-50 and 12-18·7, respectively, the tobacco plants under experiment being about seven to nine weeks old. In the case of infections under series (II), which were made between 8-15th October, but with twelve to thirteen weeks old tobacco, the percentage of positive results was 6 only.

Tobacco plants in which leaf-curl of various types was developed by experiment are illustrated in colour in Plates IX-X, showing their characteristic features in detail. Figs. 3 and 4 are of the *B* and *C* types, and Fig. 2 of the leaves of *A* type; the *A* type plant was similar to that in Fig. 1 of which the source was sann-hemp. Figs. 8 and 7 are of *X* (synthetically produced from $A+B+C+D$) and of true *X* (as occurs in nature) respectively. The differences between the appearance of the two plants are noteworthy, indicating that the composition of *X* type considered to be mixture of all types by Pal and Tandon needs accurate determination.

In 1937 three large series of transmission experiments were performed to verify the results obtained in the previous year. In the 1937 season, however, tobacco plants available for experiments were rather poor in growth and did not react to infection properly. It is widely recognised that for proper reaction to virus infection the infected plant must be growing actively [Smith, 1935]. Moreover, as plants in the field did not show leaf-curl up to the middle of October no infections could be made before that date. Particulars of the various series are given in Table IV-B.

In the first series, seventeen infections using *D* type as source were done on nine weeks old seedlings. Four to nine specimens of white-fly which had been allowed to feed on the diseased source for seven to twenty-four hours, were put on each healthy plant. In thirty-two to thirty-four days two out of seventeen infected plants developed the disease of *X* type. In the same series three plants were infected at the same time as the above, but with *X* type as source. Four to six white-flies were allowed to feed on diseased tobacco for twenty to twenty-four hours. None of the infected plants developed leaf-curl.

In the second series thirty-five infections were done in the beginning (5-9th) of November, using different types of disease as source. Only two infected (with *C* type) plants developed disease in twenty-five and thirty

days. One was almost true to the source but recovered later and the second one showed symptoms of *X* type.

The third series was performed in the end of November (29th November to 1st December). Twenty-five infections were done, but none of the infected plants developed leaf-curl.

Thus white-fly was capable of transmitting leaf-curl from diseased to healthy tobacco plants though the percentage of positive results obtained was extremely low. This, as pointed out above, was perhaps due to the poor growth of the plants under experiment as well as to the fact that the infections were done rather late in the season.

VII. *Ageratum conyzoides*, a new important host of the tobacco leaf-curl virus

In December 1936 while making survey of the weeds which showed leaf-curl it was observed that *Ageratum conyzoides*, a very common weed at Pusa, was suffering from leaf-curl with symptoms resembling those of some types of diseased tobacco. Healthy leaf and diseased plant of the weed are illustrated in detail in Plate VII, figs. 5 and 5*a*-*b*. Some transmission experiments were performed on the 16-17th December to ascertain if this plant like sann-hemp was an alternate host of the tobacco leaf-curl virus and if the white-fly could transmit the disease from *Ageratum* to tobacco. The detailed particulars of the experiments are given in Table V. Six to sixteen specimens of white-fly were fed on diseased *Ageratum* for twenty-five hours and then transferred to a healthy tobacco seedling about nine weeks old. Six plants were infected in this way. About the 18th January two infected plants showed leaf-curl of *D* type. The other four seedlings and the controls were kept under observation up to the end of the season but did not show any symptoms of leaf-curl.

In 1937 four large series of experiments were performed to verify the above results. The particulars of these experiments are also detailed in Table V, and the results are briefly summarized below :—

In the first series, forty infections were made in the first week (5-8th) of September on about eight weeks old tobacco plants. Two to sixteen white-flies were fed on diseased *Ageratum* for sixteen to twenty-five hours before they were transferred to a healthy tobacco seedling. In the course of thirteen to eighty-three days, twenty-four out of forty infected plants developed leaf-curl, and in almost all the cases the disease was of *D* type.

About the end of September (26-28th), thirty more infections (second series) were made on about six weeks old seedlings. Six to twenty-six white-flies were used per plant and they were allowed to feed on diseased *Ageratum* for twenty to twenty-five hours. In eleven to thirty-three days, eight of the infected plants developed leaf-curl, in almost all cases the type again being *D*.

The third series consisted of twenty experiments in which five to thirteen white-flies which had fed on diseased *Ageratum* for twenty-four to forty-four hours were used per plant. The infections were done in the first week of November. Between twenty-four and thirty-three days, four of the infected plants developed leaf-curl of *D* type.

The fourth and the last series of experiments consisting of thirty infections was performed on six weeks old tobacco seedlings. One to eight specimens of white-fly were used per plant. They were kept on diseased *Ageratum* for twenty-four to forty-two hours. In the course of nineteen to thirty-two days, twelve of the infected plants developed leaf-curl of typical *D* type.

From the foregoing it will be evident that *Ageratum conyzoides* is an important alternate host of the tobacco leaf-curl virus *D* and that white-fly can transmit the disease from this host to tobacco very easily. In some of the experiments described above the percentage of positive result was as high as 60. It may be recalled that Thung [1934] working in Java also successfully transmitted leaf-curl virus from this weed to tobacco with a white-fly as vector, though the highest percentage of positive infections obtained by him was about 18 only. Massop [1932] also suspected a species of *Ageratum* to be a host of tobacco leaf-curl virus in Rhodesia.

VIII. Transmission of the disease from tobacco to *Ageratum conyzoides*

Having successfully transmitted the disease from *Ageratum* to tobacco with the help of white-fly, it was natural to ascertain if the disease could be transmitted back from tobacco to *Ageratum*. Therefore about the end of October (29-30th), 1937, a series of experiments was performed for this purpose. The particulars of the experiments are detailed in Table VI. The weed which had been specially grown for experimental purposes was about eleven weeks old at the time of infection. Two to nine specimens of white-fly were allowed to feed on diseased tobacco of *D* type and then transferred to a healthy seedling of the weed. Nine infections were made in this way. In fifteen to forty-seven days all the nine infected plants developed leaf-curl resembling that which occurs in the field (Plate VIII, fig. 4).

IX. Transmission of the disease from *Ageratum conyzoides* to sann-hemp and back to the weed

Sann-hemp and *Ageratum* having definitely proved to be alternate hosts of tobacco leaf-curl, infections were made to determine the relationship of the viruses in the two alternate hosts. In the first week of September 1937, eighteen sann-hemp plants about nine weeks old were infected. Two to seven white-flies which had been allowed to feed for eighteen to twenty-three hours on diseased *Ageratum* were put on each sann-hemp plant. In seventy-two to seventy-eight days two infected plants developed leaf-curl of S.V.2 type (Table VII). In one case side shoots also showed the disease.

On 11th and 12th October two series of experiments were performed to determine if the disease could be transmitted by white-fly from sann-hemp (of both S.V.1 and S.V.2 type) to *Ageratum*. Twenty out of twenty-one infected plants of the weed developed typical leaf-curl in 38-100 days (Table VIII). In Plate VIII, figs. 2 *a-b*, photographs of some of the plants in which the disease was produced by experiment are reproduced.

X. Transmission of the virus from diseased weed (*Ageratum conyzoides*) to healthy weed

Experiments were also performed to ascertain the role of white-fly as a transmitter of the virus from diseased to healthy weed. Twenty-four infections were made during the month of October 1937, using five to eleven specimens of the white-fly per plant, which had been allowed to feed on diseased weed for about twenty-four hours. In thirteen to seventy-one days, twenty-three out of twenty-four infected plants developed leaf-curl similar to that which occurs in nature. The particulars of the experiments are given in detail in Table IX. In Plate VIII, figs. 3 *a-b*, photographs of some plants in which the disease was produced by experiment and of two control plants (figs. 3 *c-d*) are reproduced.

Thus the experiments described above clearly show that *Ageratum conyzoides* is a very important alternate host of the tobacco leaf-curl virus and that white-fly can easily transmit the virus from this weed to tobacco and sann-hemp and back from these hosts to the weed as well as from diseased to healthy weed plants.

XI. Number of white-flies and their period of feeding on diseased source necessary for the transmission of leaf-curl and the incubation period of the disease inside the plant

We have not yet performed exact experiments to ascertain the minimum number of white-flies and the period of their feeding on diseased source which are necessary for successfully transmitting leaf-curl to tobacco, sann-hemp and *Ageratum*. Similarly, no special experiments have been performed to determine the minimum and maximum incubation periods of the disease inside the infected plants at different times of the year. However, the detailed particulars of the numerous experiments which are given in Tables I-IX, and which have been summarised in the previous pages, throw some light on the factors named above. The particular experiments under various series which give information about these factors are incorporated in the statements given below. The minimum number of white-flies tried and found successful in transmitting the disease to various hosts is shown in column 5 of the statements. The minimum period of time tried for which the known number (given in column 4) of white-flies fed on diseased source and transmitted the disease is shown in column 7, the minimum and maximum incubation periods of the disease inside the various plants are stated in columns 9 and 10. It was noticed that when the number of white-flies under trial

was the minimum, the period for which they fed to become viruliferous was not necessarily the least, neither was so the incubation period. Therefore for each pair of plants (the source and the infected plant) generally four experiments give the necessary information about the four factors named above, *viz.*, minimum number of white-flies tried, minimum period of feeding tried, minimum and maximum incubation periods of the disease. In a few cases only one experiment was found to give information about more than one factor. In such cases two or three experiments give the necessary information and have only been included in the statements. In the case of crops like tobacco and sann-hemp which have more than one type of leaf-curl, information on the factors referred to above is given separately for each type. The data given in the statements are briefly summarized below :—

Transmission of the disease from sann-hemp to tobacco (Statement I)

The least number of individuals tried which successfully transmitted the disease of S.V.2 type was five. They had been kept on the diseased source for twenty-five hours and the incubation period of the disease in this case was thirty-six days which proved to be the maximum for this type. The minimum period of feeding on diseased source in the case of successful transmission was $20\frac{1}{2}$ hours, the number of specimens used being ten. A smaller number was not tried. The minimum incubation period was eleven days and was so in the case of the experiment in which the white-flies had fed for the minimum period.

With diseased sann-hemp of S.V.1 type as source, the least number of specimens tried which successfully transmitted the disease was six. The minimum period of feeding was $18\frac{1}{2}$ hours, using seventeen individuals. The maximum and minimum incubation periods were eighty-four and eight days respectively. It is noteworthy that in the case of both types the minimum incubation period of the disease inside tobacco was noted when the plants were infected towards the end of September (27-29th September, 1937).

Transmission of leaf-curl from tobacco to sann-hemp (Statement I)

Type A.—The minimum number of white-flies tried and found successful was two. They had fed for about seventeen hours. The minimum feeding period successfully tried was thirteen hours, using three specimens. The incubation period varied between 53 and 112 days, the time of infection being from the end of July up to end of August.

Statement I

Serial No.	Time and source of infection and infected plant	Type of disease of source	No. of white-flies, used	Minimum No. of white-flies tried which transmitted disease	Feeding period	Minimum feeding period tried resulting in successful transmission	Incubation period	Minimum incubation period	Maximum incubation period	Type of disease appearing and remarks
1	2	3	4	5	6	7	8	9	10	11
					Hrs.		Days	Days	Days	
	Sann-hemp to tobacco									
1	24th September 1936 .	S.V.2 .	5	5	25	...	36	...	36	*A*
2	27th September 1937 .	Do. .	10	...	20½	20½	11	11	...	*A*
3	27th September to 1st November 1937.	Do. .	7—13	...	20½—24	...	11	11	...	*A*
1	14th December 1936 and 9th September 1937.	S.V.1 .	6	6	24, 29	...	39, 63, 13	...	...	*X*
2	4th November 1936 .	Do. .	26	...	...	18½	84	...	84	*X*
3	29th September 1937 .	Do. .	13—27	...	48¾	...	8	8	...	*X*
	Tobacco to sann-hemp									
1	27th to 30th August 1937	*A* . .	2	2	16½—17	...	53, 84	53	...	S.V.2
2	21st July 1937 . .	*A* . .	3	...	13	13	...	...	112	
1	16th July 1937 .	*X* . .	6	...	6	6	117	117	...	
2	18th July 1937 . .	*X* . .	3	3	8	...	122	...	122	
	Sann-hemp to sann-hemp									
1	3rd September 1937 .	S.V.1 .	2	2	20	...	71	...	...	
2	5th November 1936 .	Do. .	24	...	19	19	31	...	...	
3	11th October 1936 .	Do. .	10	...	24	...	27	27	...	
4	13th October 1936 .	Do. .	5	...	24	...	136	...	136	

Type X.—The minimum number of white-flies tried which successfully transmitted the disease was three. They had fed for eight hours and the incubation period in that case was 122, which was the maximum for this type. The minimum feeding period tried was six hours, the number of individuals used being three.

Transmission of the disease from sann-hemp to sann-hemp (Statement I)

The minimum number of specimens tried and found successful was two. They had fed on diseased source for twenty hours. The minimum period for which white-flies were fed and transmitted disease was nineteen hours. A shorter feeding period was not tried. The incubation period varied between 27 and 136 days.

Transmission of the disease from tobacco to tobacco (Statement II)

Type A.—The least number of individuals tried and found successful was three. They had fed for eight hours. which was the minimum period tried for this type. The incubation period varied between seventeen and ninety-four days, the minimum and maximum being with the infections done in the beginning of February and during December respectively.

Type B.—The minimum number of white-flies tried and found successful was six. They had fed for twenty-four hours. The minimum feeding period tried was six hours, using six to seven individuals. The incubation period varied between 18 and 110 days, the minimum again being in the case of the infection which was done in the beginning of February.

Type C.—The minimum number of specimens and the minimum period of feeding tried and found successful were five, and five hours respectively. The incubation period varied between twelve and fifty-nine days, the minimum and maximum being in the case of the infections done in the end of October and during December respectively.

Type D.—The least number of specimens tried which transmitted the disease was four. They had fed for six hours, which was the minimum period tried for this type. The incubation period varied between twenty and ninety-four days, the minimum and maximum being in the case of the infections which were done in the beginning of February and during December respectively.

When the disease was produced synthetically by feeding the white-fly on types *A*, *B*, *C* and *D* successively, the minimum number of specimens which were tried and transmitted the disease was thirteen, the minimum feeding period tried being six hours on each type. The incubation period varied between 37 and 140 days.

From the foregoing it will be clear that as few as two flies could successfully transmit the disease. The minimum feeding period tried giving rise to successful transmission was five hours. The incubation period varied from 12 to 140 days, the minimum and maximum being generally in the case of those infections which were done in the beginning of February and during December respectively.

Statement II

Serial No.	Time and source of infection and infected plant	Type of disease of source	No. of white-flies, used	Minimum No. of white-flies tried which transmitted disease	Feeding period	Minimum feeding period tried resulting in successful transmission	Incubation period	Minimum incubation period	Maximum incubation period	Type of disease appearing and remarks
1	2	3	4	5	6	7	8	9	10	11
	Tobacco to tobacco				Hrs.		Days	Days	Days	
1	15th October 1936 .	*A* . .	3	3	8	8	26	...	...	*A* ? Source also slightly mixed.
2	6th February 1937 .	*A* . .	9	...	18	...	17	17	...	*A* ?
3	25th December 1936 .	*A* . .	5	...	18	...	94	...	94	*X*
1	1st November 1936 .	*B* . .	6	6	24	...	36	...	...	*B* ?
2	16th December 1936 and 4th January 1937.	*B* . .	6—7	...	6	6	68	...	...	*B*
3	6th February 1937 .	*B* . .	7	...	18	...	18	18	...	*B*
4	1st November 1936 .	*B* . .	11	...	24	...	110	...	110	*B*
1	31st October 1936 to 16th December 1936.	*C* . .	5	5	24—26	...	37—59	...	59	*C*
2	28th October 1936 .	*C* . .	10	...	5	5	18	...	...	*C*
3	31st October 1936 .	*C* . .	7	...	24	...	12	12	...	
1	26th December 1936 and 14th October 1937.	*D* . .	5	4	6—7	6	94, 34	...	94	*D* ?
2	6th February 1937 .	*D* . .	7	...	18	...	20	20	...	*D*
1	16th October 1936 .	*X* . .	2	2	$7\frac{1}{2}$	$7\frac{1}{2}$	25	...	25	*X*
2	8th-9th February 1937.	*X* . .	5	...	24	...	11	11	...	*X*
1	27th December 1936 .	*A*+*B*+*C*+*D*	13	13	6	6	52	...	...	*X*
2	Ditto . .	Do. .	21	...	6	...	37	37	...	*X*
3	5th November 1936 .	Do. .	26	...	21	...	140	140	140	*X*

Statement III

Serial No.	Time and source of infection and infected plant	Type of disease of source	No. of white-flies used	Minimum No. of white-flies tried which transmitted disease	Feeding period	Minimum feeding period tried resulting in successful transmission	Incubation period	Minimum incubation period	Maximum incubation period	Type of disease appearing and remarks
1	2	3	4	5	6	7	8	9	10	11
	Ageratum to tobacco				Hrs.		Days	Days	Days	
1	22nd November 1937	...	1	1	24	...	19	...	...	*D*
2	7th -8th September 1937	...	5—16	...	16	16	13—83	...	...	*D*
3	Ditto	...	8	...	16	...	13	13	...	*D*
4	Ditto	...	11	...	16	...	83	...	83	*D*
	Tobacco to Ageratum									
1	29th and 30th October 1937	*D*	2—9	2	16	16	15—47	...	...	Resemble that in the field.
2	Ditto	...	...	...	...	...	15	15	...	
3	Ditto	...	...	...	...	...	47	...	47	
	Ageratum to sann-hemp									
1	3rd September 1937	...	3	3	23	...	78	...	78	S. V. 2
2	4th September 1937	...	5	...	18	18	72	72	...	S. V. 2.
	Sann-hemp to Ageratum									
1	11th-12th October 1937	S. V. 1	5—12	5	24	24	49—69	...	...	
2	Ditto	Do.	...	...	...	...	49	49	...	
3	Ditto	Do.	...	...	...	...	69	...	69	Type resembles that in the field.
1	Ditto	S. V. 2	5—11	5	24	24	38—100	...	...	
2	Ditto	Do.	...	...	24	...	38	38	...	
3	Ditto	Do.	...	...	24	...	100	...	100	
	Ageratum to Ageratum	...	5—11	5	24	...	13—71	...	...	
1	10th and 11th October 1937	...	...	...	...	...	13	13	...	
2	Ditto	...	...	...	...	...	71	...	71	
3	29th-31st October 1937.	...	6—10	...	22—24	22	27—39	...	...	

Transmission of the disease from Ageratum *to tobacco, sann-hemp,* Ageratum *etc.* (*Statement III*)

Even one white-fly which had been fed for twenty-four hours on diseased *Ageratum* successfully transmitted the disease to tobacco. However, the minimum feeding period successfully tried was sixteen hours, using five individuals. The incubation period varied between thirteen and eighty-three days.

In the case of tobacco to *Ageratum*, the minimum number of individuals successfully tried was two. They had fed for sixteen hours.

The minimum incubation period with tobacco and *Ageratum* as host and source respectively and *vice versa* was generally thirteen to fifteen days. In the case of *Ageratum* and sann-hemp, however, it was thirty-eight to forty-nine days.

XII. Other food plants of white-fly, *Bemisia gossypiperda* M. & L.

In addition to tobacco, *Ageratum conyzoides* and sann-hemp mentioned in the previous pages, white-fly has been found feeding and breeding on a variety of plants, both cultivated and wild, in the neighbourhood of Pusa. The list of various plant hosts is given below, and those which have been observed to suffer from some kind of leaf-curl have been marked with an asterisk :—

Cultivated plants

* *Althaea rosea* (hollyhock)
Cannabis sativa (*ganja*)
Hibiscus cannabinus
H. esculentus (lady's fingers)
Gossypium herbaceum (cotton)
* *Cucumis sativa* (cucumber)
C. melo (melon)
* *Lagenaria vulgaris* (bottle gourd)
Trichosanthes dioica
**Lycopersicum esculentum*
Nicotiana glauca
N. rustica
N. glutinosa
Capsicum annuum
Solanum tuberosum (potato)
S. melogena (brinjal)
Glycine hispida (soya bean)
* *Phaseolus calcaratus*
P. radiatus (green gram)
P. mungo (black gram)
Medicago sativa (lucerne)
Trifolium alexandrinum (berseem)
* *Zinnia elegans*
* *Petunia* sp.

Wild plants

Hibiscus rosasinensis (shoe flower)
* *Sida rhombifolia*
Nicotiana plumbaginifolia (wild tobacco)
**Solanum nigrum*
S. verbasiofolium
* *Datura stramonium* (datura)
Launea asplenifolia
Duranta plumieri (duranta)
Euphorbia hirsuta
Anisomeles ovata (wild bur)
Nepeta ruderalis
* *Scoparia dulcis*
Vernonia cinerea var. anthelmentica
Ocimum sanctum
Ocimum basilicum.

Cultivated plants

Arachis hypogea (groundnut)
Cajanus cajan (red gram)
Luffa acutangula (ribbed gourd)
Cucurbita maxima (pumpkin)

As is well known this white-fly is a serious pest of cotton in the Punjab. Husain and Trehan [1936] have given a complete list of its host plants in that province. However, this white-fly is not reported to cause leaf-curl in cotton or tobacco in the Punjab. Whereas cotton has been carefully examined for leaf-curl, a survey of tobacco areas for the incidence of leaf-curl is warranted, as one of us (H. S. P.) has recently observed a crop of tobacco badly affected by leaf-curl of all types near Agra in the western United Provinces.

XIII. Discussion and conclusions

From the observations described in the previous pages the following conclusions can be drawn :—

Sann-hemp is an important alternate host of the tobacco leaf-curl viruses *A* and *X*, the two viruses causing different symptoms in sann-hemp, giving rise to two types of disease, which have been designated as S. V. 1 and S. V. 2 in the paper. White-fly, *Bemisia gossypiperda*, can easily transmit both types of disease from sann-hemp to tobacco.

Though white-fly can transmit leaf-curl from tobacco back to sann hemp, it does not however seem to be a very efficient vector in this respect Moreover, in view of the fact that the times of growth of the two crops are different, tobacco does not seem to serve as a source of infection for the disease in sann-hemp.

The weed *Ageratum conyzoides* is another important alternate host of tobacco leaf-curl, especially of *D* type. White-fly is capable of easily transmitting the disease from this host to tobacco. White-fly can also easily transmit the disease from tobacco back to the weed. Sometimes 100 per cent positive transmissions were obtained. White-fly is capable also of easily transmitting the disease from sann-hemp to *A. conyzoides*, though it cannot transmit the disease back to sann-hemp so easily.

Regarding the role of white-fly as a vector of leaf-curl from diseased to healthy tobacco, the results of two years' experiments show that white-fly could transmit the disease only in a few cases. Therefore, it appears that in nature diseased tobacco is not an important source of infection for healthy tobacco, and that the alternate hosts referred to above, *viz.*, sann-hemp and *A. conyzoides* seem to play a much more important part in this connection. These two hosts harbour leaf-curl viruses *A*, *D* and *X* types ; the alternate hosts, if any, for *B* and *C* types remain yet to be discovered. In Java also it has been shown [Thung, 1932 ; 1934] that the chief source of infection to tobacco lies outside the crop.

It appears that tobacco is most susceptible to getting the infection if it is about eight weeks old and if the infection takes place in autumn or in February. Sann-hemp is most susceptible during July and August, when

it is about two to three months old. Wille [1928] working on ' leaf-crinkle ' of sugar beet also concluded that the disease is very much influenced by the age of the host plant, while Spencer [1938] has recently observed seasonal variations in susceptibility of tobacco to infection with tobacco-mosaic virus. Regarding the autumn and spring seasons being more suitable than winter for the infection of tobacco *, it is likely that the air temperature and humidity prevailing during these seasons are conducive to the development of the disease. In this respect the case of tobacco leaf-curl seems to be similar to that of mosaic disease of some Cruciferae in the United States, which is very severe if the temperature is 70°-80° F., whereas at 55°-65°F. even more susceptible hosts cease to show mosaic symptoms. It may also be recalled that ' yellow dwarf ' of potatoes in America develops severe symptoms at 25°C., while no symptoms appear at 15°C. [Goss and Peltier, 1925]. The ' curly top ' of sugar beet also behaves similarly [Smith, 1933]. Grainger [1936] has recently observed the same phenomenon in the case of tobacco mosaic. However, there are numerous cases on record [Smith, 1933] in which opposite is the case, *viz.*, low temperatures accentuate the symptoms of the disease, whereas higher temperatures suppress them.

The incubation period of the disease inside the infected plant seems to be connected with the question of the accentuation or suppression of the symptoms of the disease discussed above. It was noticed that with the age of infected tobacco remaining the same, the incubation period was generally minimum if the infections were done during September and October or in February when the air temperature starts rising at Pusa. The incubation period was generally the maximum if the infections were done during December. Spencer [1938] has recently found the same in the case of tobacco-mosaic virus. Apart from air temperature affecting the plant, it is quite likely that white-fly is most viruliferous at these times of the year, which again may be due to several environmental factors including temperature and humidity. Storey [1933] attached great importance to the influence of weather condition on the ability of the insect vector to take up and transmit viruses.

We have not performed exact experiments to determine the length of time necessary for white-fly to feed on the diseased host for successfully transmitting the disease to healthy host, but the general transmission experiments show that white-fly can transmit the virus after five to six hours' feeding, shorter periods not having been tested. Kirkpatrick [1931] found this period to be about $4\frac{1}{2}$ hours in the case of the white-fly (*Bemisia* sp.) which spreads leaf-curl of cotton in Sudan. Storey [1928] showed that the leaf-hopper *Cicadulina mbila* could acquire the maize streak virus after one hour's feeding, whereas Doolittle and Walker [1928] concluded that *Aphis gossypii* could pick up the cucumber mosaic virus after feeding for five minutes only. Recently, Watson [1936] has obtained the highest proportion of infection after two to five minutes feeding by *Myzus persicae* on the infected plants.

* We could not perform any experiment during the hottest season of the year (April-June) as tobacco crop is not grown at that time in Bihar.

PLATE VII

FIG. 1. Sann-hemp plant suffering from leaf-curl of S. V. 1 type

FIG. 2. Sann-hemp plant suffering from leaf-curl of S. V. 2 type

FIG. 2-*a* Leaf of the same, magnified

FIG. 3. A leaf of a typical sann-hemp plant, free from leaf-curl, magnified

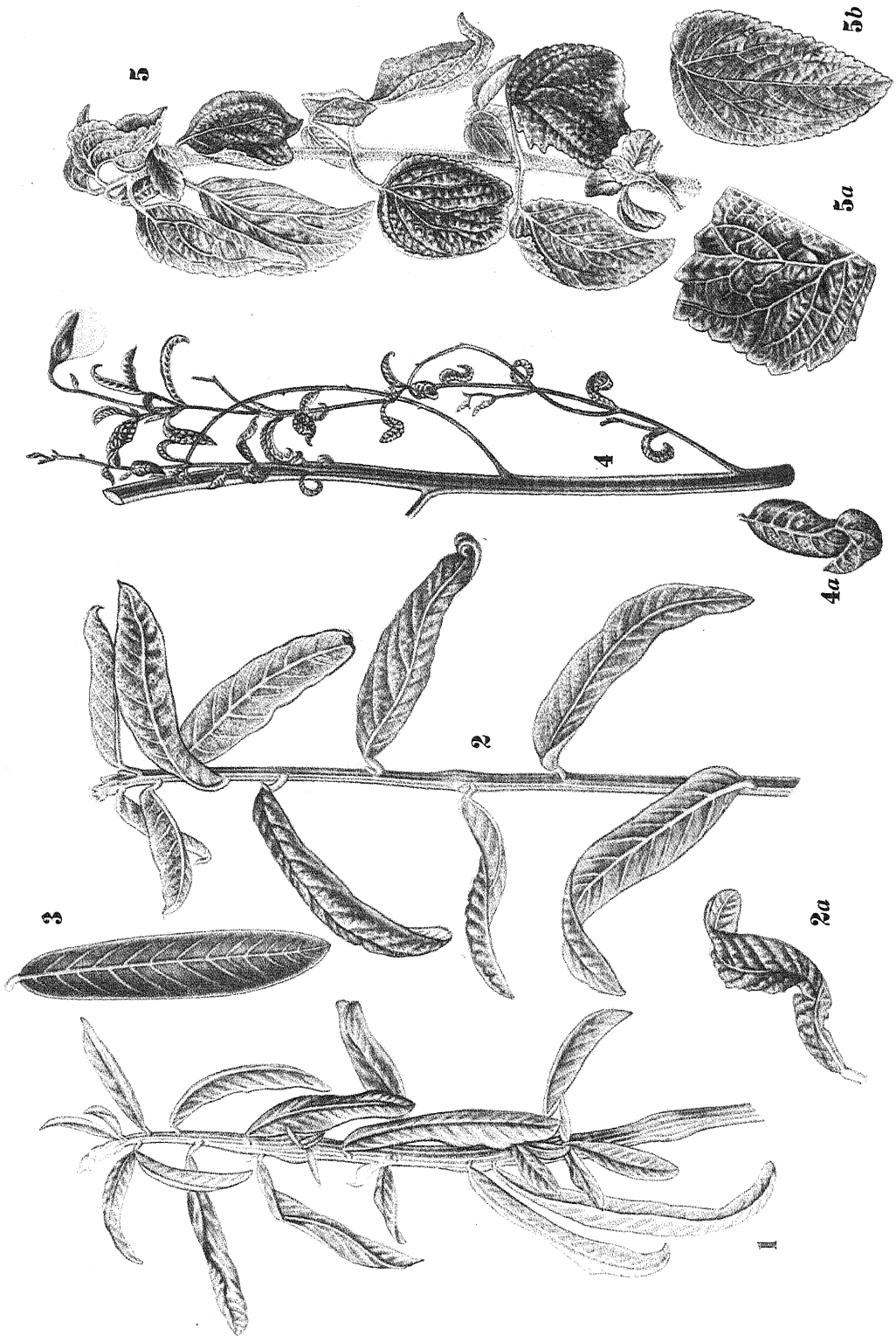
FIG. 4. Sann-hemp plant in which leaf-curl was produced by experiment with diseased tobacco (*A* type) as source of infection

FIG. 4-*a* Leaf of the same, magnified

FIG. 5. A twig of *Ageratum conyzoides* suffering from leaf-curl

FIG. 5-*a* A leaf of the same, enlarged

FIG. 5-*b* A leaf from healthy twig


5
5b
5a
4
4a
2
2a
3
1

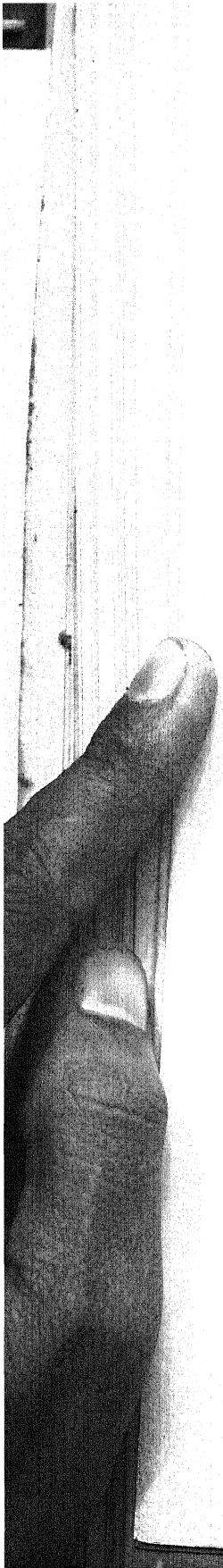
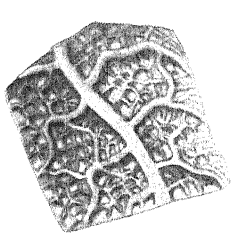


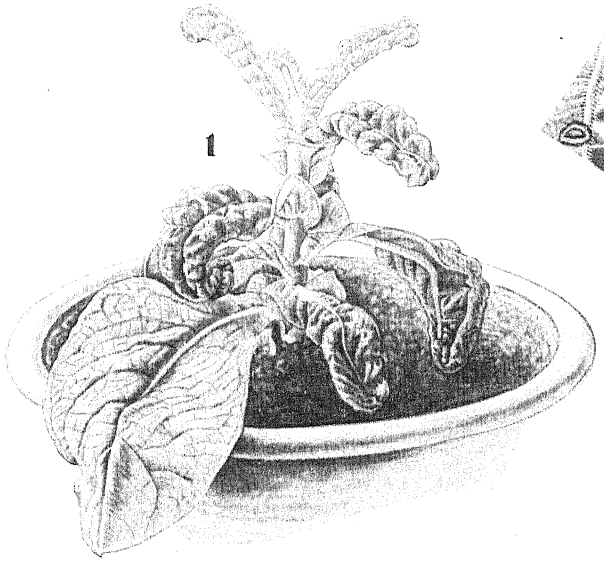
PLATE VIII

1. A series of tobacco plants (a—h) in which leaf-curl was produced by experiment with diseased sann-hemp as source of infection, j & k two typical control plants
2. Photos of some *Ageratum* plants (a—b) in which leaf-curl was produced by experiment, with diseased sann-hemp as source of infection
3. Photos of some *Ageratum* plants (a—b) in which leaf-curl was produced by experiment, with diseased *Ageratum* as source of infection ; plants c and d are controls of these as well as of the plants in Fig. 2 above
4. Photo of an *Ageratum* plant in which leaf-curl was produced by experiment, with diseased tobacco as source of infection.

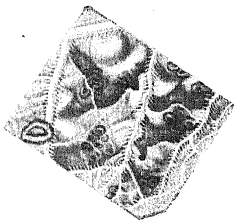
1
a
b
c
d
e
f
g
h
j
k
2
a
b
3
a
b
c
d
4



2

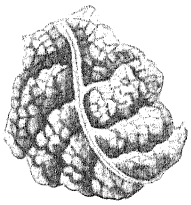


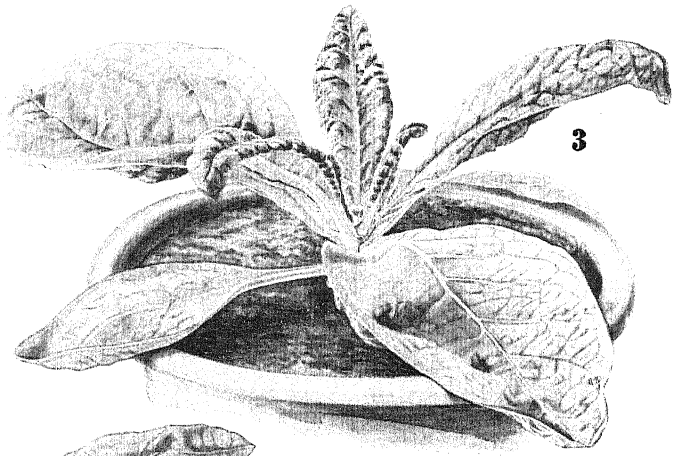
1



1*a*

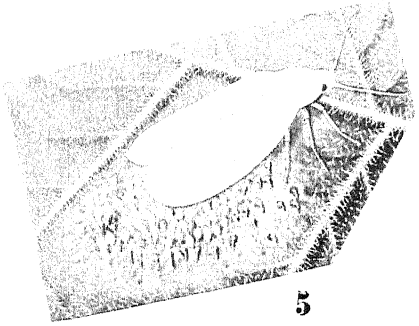
2*a*





3

4



5

PLATE IX

1. A tobacco plant in which leaf-curl of *A* type was produced by experiment with diseased sann-hemp as source of infection

1-*a*. Lower side of a leaf of the same—note the cup-shaped enations

2 & 2*a*. Lower and upper sides of the leaves of a tobacco plant in which leaf-curl of *A* type was produced using similarly diseased tobacco plant as source of infection

3. A tobacco plant in which leaf-curl of *B* type was produced by experiment with similarly diseased tobacco plant as source of infection

4. A tobacco plant in which leaf-curl of *C* type was produced by experiment with similarly diseased tobacco plant as source of infection

5. The white-fly, *Bemisia gossypiperda*, laying eggs on a tobacco leaf

PLATE X

6. A tobacco plant in which leaf-curl of *D* type was produced by experiment with similarly diseased tobacco plant as source of infection

6-*a*. A leaf of the same, magnified

7. A tobacco plant in which leaf-curl of *X* type was produced, with a similarly affected tobacco plant as source of infection

8. A tobacco plant in which leaf-curl was produced by experiment with tobacco plants suffering from *A*, *B*, *C* and *D* types as source of infection

8-*a*. Lower surface of a leaf of the plant in FIG. 3, magnified (Note the prominent enations)

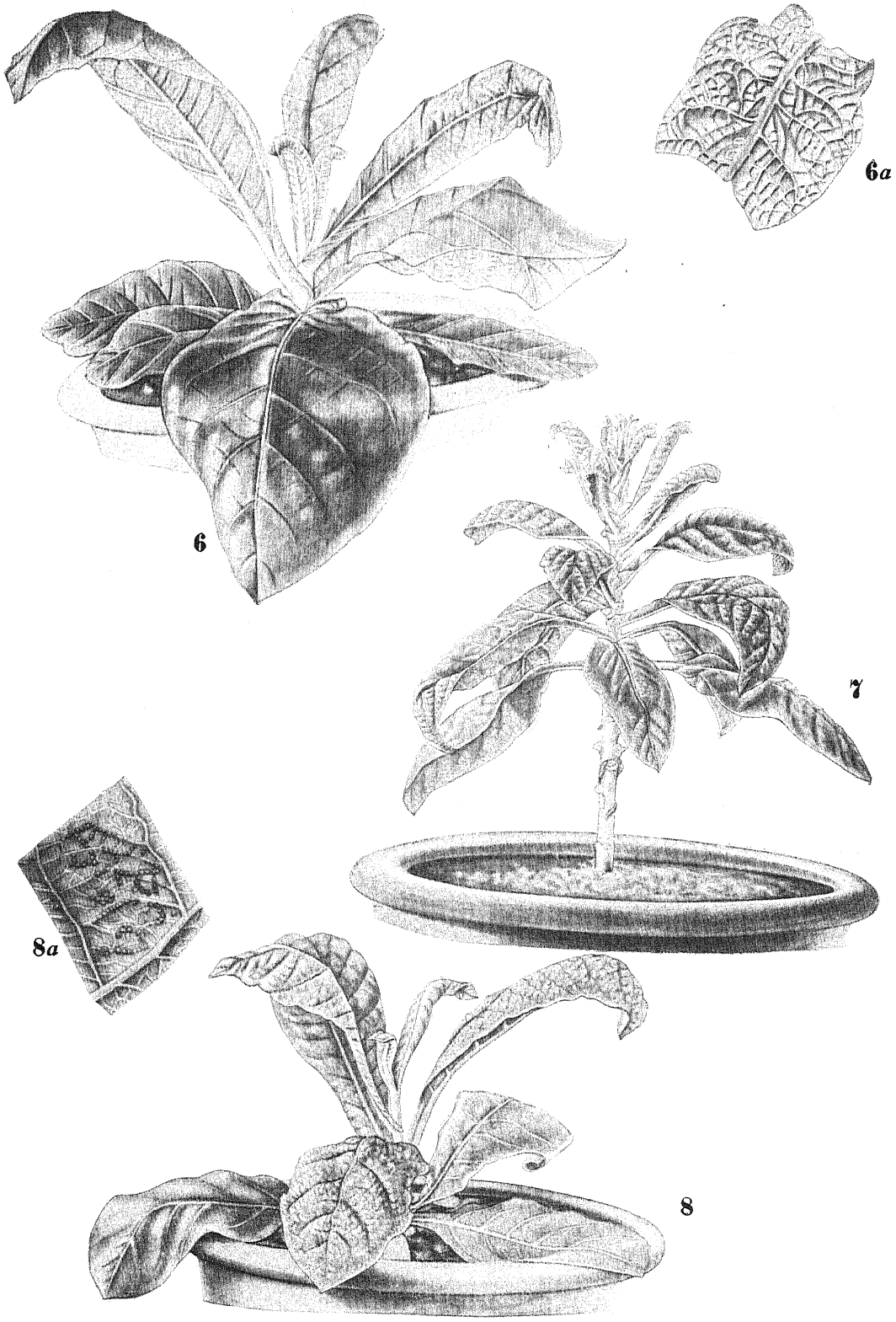

6a
6
7
8a
8

TABLE I-A

Particulars of experiments performed to transmit the disease from sann-hemp to tobacco during 1936

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks
1	2	3	4	5	6	7	8
Series i				(Hours)		(Days)	
Time of experiment, 6th-12th September 1936	1—3	...	6th Sept. 1936—8 A.M.	24	3—5	...	Negative.
Age of diseased sann-hemp (Source) : 8-10 weeks.	4—7	...	7th-12th Sept. 1936—8 A.M.	48	2—4	...	Do.
Age of healthy tobacco infected : about 8 weeks.							
Series ii							
Time of experiment, 20th-29th September 1936	1	...	23rd Sept. 1936—10-30 A.M.	25	5	36	Positive ; type *A*.
Age of diseased sann-hemp (Source) : 11-12 weeks.	2—4	...	20th-23rd Sept. 1936—10-30 A. M.	24—52½	2—5	...	Negative.
Age of healthy tobacco infected : 10-11 weeks.	5—8	...	28th Sept. 1936—5-45 P.M.	17¾	3—12	...	Do.
Series iii							
Time of experiment, 3rd-5th November 1936	1	...	3rd Nov. 1936—10 A.M.	24	9	19	Positive ; type *A*.
Age of diseased sann-hemp (Source) : 17 weeks.	2—6	...	4th Nov. 1936—4 P.M.	18½	11—21	...	Negative.
Age of healthy tobacco infected : 7 weeks.	7	...	Do. . .	18½	26	84	Positive ; type *X*.
	8	...	Do. . .	18½	23	74	Do.
	9	...	Do. . .	18½	17	17	Do.
	10	...	Do. . .	18½	18	17	Do.

TABLE I-A—*contd.*

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks
1	2	3	4	5	6	7	8
				(Hours)		(Days)	
Series iv							
Time of experiment, 3rd-4th November 1936.	1—7	...	3rd Nov. 1936—11 A.M.	24	7—17	...	Negative.
Age of diseased sann-hemp (Source) : 17 weeks.	...	...	...	...	...	...	
Age of healthy tobacco infected : 16 weeks.							
Series v							
Time of experiment, 14th December 1936	1	...	14th Dec. 1936—10 A.M.	24	6	39	Positive ; type *X*.
Age of diseased sann-hemp (Source) : 22 weeks.	2	...	Do. . .	24	6	63	Positive, A type *X*.
Age of healthy tobacco infected : about 8 weeks.	3—10	...	Do. . .	24	3—7	...	Negative.

None of the controls of the various series developed leaf-curl disease

TABLE I-B

Particulars of experiments performed to transmit the disease from sann-hemp to tobacco during 1937

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks
1	2	3	4	5	6	7	8
				(Hours)		(Days)	
Series i							
Time of experiment, 9th-11th Sept. 1937	1	S. V. I .	9th Sept. 1937—10-30 A.M.	29	14	51	Positive ; type *X*
Age of diseased sann-hemp (Source) : 13 weeks.	2	Do. . .	Do. . .	29	8	22	Positive ; type *D* ?
Age of healthy tobacco infected : 8 weeks.	3	Do. . .	Do. . .	29	7	62	Positive ; type *X*
	4	Do. .	Do. . .	29	10	28	Do.
	5	Do. . .	Do. . .	29	7	40	Do.
	6	Do. . .	Do. . .	29	8	40	Do.
	7	Do. . .	Do. . .	29	7	18	Do.
	8	Do. . .	Do. . .	29	10	18	Do.
	9	Do. . .	Do. . .	29	10	22	Positive ; type *D* ? (Recovered).
	10	Do. . .	Do. . .	29	7	22	Positive ; type *X*.
	11	Do. . .	Do. . .	29	11	18	Do.
	12	Do. . .	Do. . .	29	6	13	Do.
	13—27	Do. . .	10th Sept. 1937—4 P.M.	24	6—13	...	Negative.
	28	Do. . .	Do. . .	24	9	17	Positive ; type *X* ? (Recovered)
	29	Do. . .	Do. . .	24	11	17	Do.
	30—32	Do. . .	Do. . .	24	8—9	...	Negative.

TABLE I-B—*contd.*

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks
1	2	3	4	5	6	7	8
				(Hours)		(Days)	
Series i—contd.	33	S. V. I.	10th Sept. 1937—4 P.M.	24	7	17	Positive; type *X* (Recovered).
	34—39	Do. . .	Do. . .	24	6—14	...	Negative.
	40	Do. . .	Do. . .	24	10	32	Positive; type *X*.
Series ii							
Time of experiment, 27th September to 1st October 1937	1	S. V. 1 .	27th Sept. 1937—11 A.M.	20½	19	11	Positive; type *X*.
Age of diseased sann-hemp (Source): about 15 weeks.	2	Do . .	Do. . .	20½	21	15	Do.
	3	Do. . .	Do. .	20½	17	11	Do.
Age of healthy tobacco infected: about 7 weeks.	4	Do. . .	Do. . .	20½	11	...	Negative.
	5	Do. . .	Do. . .	20½	9	...	Do.
	6	Do. . .	Do. . .	20½	14	15	Positive; type *X*.
	7	Do. . .	Do. . .	20½	8	11	Do.
	8	Do. . .	Do. . .	20½	11	11	Do.
	9	Do. . .	29th Sept. 1937—10 A.M.	48¾	11	12	Do.
	10	Do. . .	Do. . .	48¾	25	8	Do.
	11	Do. . .	Do. . .	48¾	22	12	Do
	12	Do. . .	Do. . .	48¾	21	11	Do.
	13	Do. . .	Do. . .	48¾	16	...	Negative.
	14	Do. . .	Do. . .	48¾	17	11	Positive; type *X*.

	15	Do. . .	Do. . .	48¾	13	8	Do.
	16	Do. . .	Do. . .	48¾	12	...	Negative.
	17	Do. . .	Do. . .	48¾	23	11	Positive ; type *X*.
	18	Do. . .	Do. . .	48¾	22	11	Do.
	19	Do. . .	Do. . .	48¾	9	...	Negative.
	20	Do. . .	Do. . .	48¾	24	11	Positive ; type *X*.
	21	Do. . .	Do. . .	48¾	23	8	Do.
	22	Do. . .	Do. .	48¾	18	14	Do.
	23	Do. . .	Do. . .	58¾	14	11	Do
	24	Do. . .	Do. .	48¾	19	12	Do.
	25	Do. . .	Do. .	48¾	26	8	Do.
	26	Do. . .	Do. . .	48¾	27	8	Do.
	27	Do. . .	Do. . .	48¾	14	25	Do.
Seriesiii							
Time of experiment, 27th-29th September 1937.	1	S. V. 2 .	27th Sept. 1937—11 A.M.	20½	10	11	Positive ; type *A*.
Age of diseased sann-hemp (Source) : about 15 weeks.	2	Do. . .	Do. . .	20½	13	...	Negative.
Age of healthy tobacco infected : about 7 weeks.	3	Do. . .	29th Sept. 1937—10 A.M.	48¾	27	12	Positive ; type *A*.
Series iv							
Time of experiment, 31st Oct.-2nd November 1937.	1	S. V. 2 .	31st Oct. 1937—Noon.	29	25	19	Positive ; type *A*.
Age of diseased sann-hemp (Source) : 18 weeks.	2	Do. . .	Do. . .	29	5	...	Negative.
Age of healthy tobacco infected : 7 weeks.	3	Do. . .	Do. . .	29	9	13	Positive ; type *A*.
	4	Do. . .	Do. . .	29	8	19	Do.
	5	Do. . .	Do. . .	29	6	...	Negative.
	6	Do. . .	Do. . .	29	11	13	Positive ; type *A*.
	7	Do. . .	Do. . .	29	7	12	Do.

TABLE I-B—*concld.*

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks
1	2	3	4	5	6	7	8
				(Hours)		(Days)	
Series iv—contd.	8	S. V. 2.	1st Nov. 1937—11 A.M.	24	6	...	Negative.
	9	Do. . .	Do. . .	24	16	...	Do.
	10	Do. . .	Do. . .	24	7	11	Positive ; type *A*.
	11—15	Do. .	Do. . .	24	5—16	...	Negative.
	16	Do. .	Do. .	24	10	16	Positive ; type *A*.
	17	Do. . .	Do. . .	24	7	21	Do.
	18	Do. . .	Do. . .	24	9	...	Negative.
	19—22	Do. . .	1st Nov. 1937 4 P.M.	24	11—13	11	Positive ; type *A*.
	23—25	Do. . .	Do. . .	24	7—14	14—15	Do.
	26	Do. . .	Do. . .	24	11	...	Negative.
	27	Do. . .	Do. . .	24	9	...	Do.
Series v							
Time of experiment, 25th-28th November 1937.	1	S. V. 2 .	25th Nov. 1937—11 A.M.	24	6	27	Positive ; type *A*.
Age of diseased sann-hemp (Source) : 23 weeks.	2—25	Do. . .	25th-28th Nov. 1937.	24	3—7	...	Negative.
Age of healthy tobacco infected : 6 weeks.							

None of the controls of the various series developed leaf-curl disease.

TABLE II-A

Particulars of experiments performed to transmit the disease from tobacco to sann-hemp during 1936

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks
1	2	3	4	5	6	7	8
Series i				(Hours)		(Days)	
Time of experiment 12th-22nd September 1936	1—3	*A*	12th-14th Sept. 1936—4 P.M.	24—48	3—10	...	Negative.
Age of diseased tobacco (Source): 13—14 weeks. Age of sann-hemp inoculated: 9—10 weeks.	4—5	*C*	21st-22nd Sept. 1936—5 P.M.	24	3—5	...	Do.
Series ii							
Time of experiment: 24th Sept.—3rd Oct. 1936	1—7	*X*	24th Sept.-3rd Oct. 1936.	17—24	1—8	...	Do.
Age of diseased tobacco (Source): 13—14 weeks. Age of sann-hemp inoculated: 10—12 weeks.	8—11	*C*	29th-30th Nov. 1936—5 P.M.	24	8—12	...	Do.
Series iii.							
Time of experiment, 13th October 1936	1—3	*B*	13th Oct. 1936—9 A.M.	8	2—7	25	Slight reaction perceptible The lower three leaves of inoculated plant were curled and drooping.
Age of diseased tobacco (Source): about 13 weeks. Age of sann-hemp inoculated: 13 weeks.	4—6	*B*	Do.	8	2—7	...	Negative.
Series iv							
Time of experiment, 5th-6th November 1936	1—8	*C*	5th-6th Nov. 1936	8—22	7—31	...	Do.
Age of diseased tobacco (Source): 17 weeks. Age of sann-hemp inoculated: 16 weeks.	9—10	*A*	Do.	22	11—13	...	Do.

None of the controls in the various series developed leaf-curl disease.

TABLE II-B

Particulars of experiments performed to transmit the disease from tobacco to sann-hemp during 1937

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks
1	2	3	4	5	6	7	8
				(Hours)		(Days)	
Series i							
Time of experiment, 16th-17th July 1937	1—4	*A*	16th-17th July 1937	16	2—4	...	Negative.
Age of diseased tobacco (of 1936-37 season as Source): 44 weeks.	5—9	*X*	Do.	16	2—4	...	Do.
	10	*C*	Do.	16	4	...	Do.
Age of healthy sann-hemp inoculated : 2½ weeks.	11	*X* of *C*	Do.	4	5	...	Do.
	12	*C* & *D*	Do.	4	5	...	Do.
	13	*X*	Do.	6	6	117	Positive ; type S. V. I.
	14	*X*	Do.	6	6	...	Negative.
	15—16	*C*	Do.	6	6	...	Do.
	17	*A*	Do.	4	5	...	Do.
Series ii							
Time of experiment, 18th-23rd July 1937.	1	*C*	18th July 1937—8 A.M.	8	3	...	Do.
Age of diseased tobacco (1936-37 season as Source) : 36 weeks.	2	*C*	Do.	8	3	119	Positive ; type S. V. I.
	3	*C*	Do.	10	7	...	Negative.
Age of healthy sann-hemp inoculated : 2½—3 weeks.	4	*A*	Do.	8	3	...	Do.
	5	*X*	Do.	8	3	122	Positive ; type S. V. I.
	6	*X*	Do.	10	7	...	Negative.

	7	*X*	Do. .	10	7	118	Positive ; type S. V. I.
	8	*X*	Do. .	10	7	...	Negative.
	9	*A*	21st July 1937—6 P.M.	13	3	...	Do.
	10	*A*	Do. .	13	3	112	Positive ; type S. V. 2.
	11—20	*A*	Do. .	13	3	...	Negative.
	21	*C*	23rd July 1937—9 A.M.	8	4	...	Do.
	22	*C*	Do. .	8	4	...	Do.
Series iii.							
Time of experiment, 23rd-29th July 1937.	1—3	*C*	23rd July 1937—9 A.M.	8	4	...	Negative.
Age of diseased tobacco (1936-37 Season as Source) : 32—40 weeks.	4	*C*	Do. .	8	4	133	Positive ; type S. V. I.
	5	*C*	Do. .	8	4	...	Negative.
Age of healthy sann-hemp infected : 3—4 weeks.	6	*C*	Do. .	8	4	131	Positive ; type S. V. I.
	7—12	*A*	24th-26th July 1937.	6—18	2—5	...	Negative.
	13—16	*X*	26th July 1937	6	4—5	...	Do.
	17—20	*A*	27th July 1937—11 A.M.	6	4—9	...	Do.
	21	*D*	29th July 1937—11 A.M.	7	4	...	Do.
	22	*D*	Do. .	7	3	...	Do.
	23	*D*	Do. .	7	3	...	Do.
	24	*C*	Do. .	7	1	...	Do.
	25	*C*	Do. .	7	3	117	Positive ; type S. V. I.

TABLE II-B—*contd.*

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks
1	2	3	4	5	6	7	8
				(Hours)		(Days)	
Series iv							
Time of experiment, 27th-31st August 1937.	1—2	*C*	27th Aug. 1937—5 P.M.	17	3	...	Negative.
Age of diseased tobacco (1936-37 season as Source): 49—50 weeks.	3	*C*	Do.	17	4	144	Positive ; type S. V. I.
	4	*C & D*	Do.	17	2	68	Do.
Age of healthy sann-hemp infected : 8—8½ weeks.	5—6	*C & D*	Do.	17	2—4	...	Negative
	7	*A*	Do.	17	3	53	Positive ; type S. V. 2
	8	*A*	Do.	17	2	53	Do.
	9—10	*X*	Do.	17	2—4	...	Negative.
	11—13	*C & D*	28th Aug. 1937—11 A.M.	6	3—4	...	Do.
	14—16	*A*	Do.	6	3—5	...	Do.
	17	*A*	30th Aug. 1937—5 P.M.	16½	3	76	Positive ; type S. V. 2.
	18	*A*	Do.	16½	2	...	Negative.
	19	*A*	Do.	16½	4	70	Positive ; type S. V. 2.
	20	*A*	Do.	16½	2	84	Do.
	21—22	*A*	Do.	16½	3—4	...	Negative.
	23	*A*	Do.	16½	5	70	Positive ; type S. V. 2.
	24	*A*	Do.	16½	4	...	Negative.

None of the controls of the various series developed leaf-curl disease.

TABLE III-A

Particulars of experiments performed to transmit the disease from sann-hemp to sann-hemp during 1936

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks
1	2	3	4	5	6	7	8
				(Hours)		(Days)	
Series i							
Time of experiment, 8th-13th October 1936	1	...	8th Oct. 1936—5-30 P.M.	24	4	57	Positive; almost similar to the source. Side shoots also were diseased.
Age of diseased sann-hemp (Source): 14—15 weeks.	2—6	...	Do. .	24	5—9	...	Negative.
Age of healthy sann-hemp infected: 13—14 weeks.	7	...	11th Oct. 1936—2 P.M.	24	10	29	Positive; almost similar to the source. Side shoots also were diseased.
	8	...	Do. .	24	10	27	Ditto.
	9—18	...	Do. .	24	10—12	...	Negative.
	19	...	12th-13th Oct. 1936—11 A.M.	24	5	136	Positive; almost similar to the source, side shoots also were diseased.
	20—24	...	Do. .	24	5—7	...	Negative
Series ii							
Time of experiment, 4th/5th November 1936	1	...	4th-5th Nov. 1936—4 P.M.—11 A.M.	19	24	31	Positive; almost similar to the source, side shoots also were diseased.
Age of diseased sann-hemp (Source): 17 weeks.							
Age of healthy sann-hemp infected: 17 weeks.	2—10	...	Do. .	19	11—27	...	Negative.

None of the controls of the various series developed leaf-curl disease.

TABLE III-B

Particulars of experiments performed to transmit the disease from sann-hemp to sann-hemp during 1937

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks.
1	2	3	4	5	6	7	8
				(Hours)		(Days)	
Series i							
Time of experiment, 2nd-3rd September 1937	1	...	2nd-3rd Sept. 1937 —NOON.	20	2	71	Positive ; type S. V. I.
Age of diseased sann-hemp (Source) : 12 weeks.	2	...	Do.	20	3	79	Ditto
Age of healthy sann-hemp infected : 9 weeks.	3—8	...	Do.	20	2—5	...	Negative.
Series ii							
Time of experiment, and other details are same as in series *i*.	1	...	Do.	20	3	67	Positive ; type S. V. 2.
	2	...	Do.	20	3	72	Ditto.
	3—9	...	Do.	20	2—6	...	Negative.

None of the controls in the various series developed leaf-curl disease

TABLE IV-A

Particulars of experiments performed to transmit the disease from tobacco to tobacco during 1936

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks.
1	2	3	4	5	6	7	8
				(Hours)		(Days)	
Series i							
Time of experiment 14th-29th September 1936	1—6	*C*	14th-19th Sept. 1936—4 P.M.—8 A.M.	16—48	5—12	...	Negative.
Age of diseased tobacco (Source) : 14—16 weeks.	7	*C*	19th Sept. 1936—5 P.M.	48	5	39	Positive ; type *C*.
	8	*C*	Do.	48	3	...	Negative.
	9—12	*X*	18th Sep. 1936—4-30 P. M.	16	1—2	...	Do.
Age of healthy tobacco infected : 9—11 weeks.	12—13	*X*	28th Sept. 1936—5 P.M.	17	2	...	Do.
	14	*X*	Do.	17	3	31	Positive ; type *X*.
	15—17	*B*	25th Sept. 1936—4 P.M.	19	5	...	Negative.
Series ii							
Time of experiment, 8th-15th October 1936	1—7	*C*	8th-13th Oct. 1936—4 P.M.—5 P.M.	8—24	3—6	...	Negative.
Age of diseased tobacco (Source) : 12—13 weeks.	8—12	*B*	8th-10th Oct. 1936—4 P.M.—5 P.M.	24	1—7	...	Do.
Age of healthy tobacco infected : 12—13 weeks.	13	**A*	15th Oct. 1936—8 A.M.	8	3	26	Positive ; type *X*. *Type A (Source) contained a little mixture of *C* & *D*.
	14—17	*A*	Do.	8—9	4—5	...	Negative.

TABLE IV-A—*contd.*

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks
1	2	3	4	5	6	7	8
				(Hours)		(Days)	
Series iii							
Time of experiment, 16th-17th Oct. 1936	1	*X*	16th Oct. 1936—9 A.M.	7½	2	25	Positive; type *X*.
Age of diseased tobacco (Source): 9 weeks.	2—3	*X*	Do.	7½	3—5	...	Negative.
Age of healthy tobacco infected: 9 weeks.	4	*X*	17th Oct. 1936—9 A.M.	8	5	22	Positive; type *X*.
Series iv							
Time of experiment, 28th October-6th November 1936	1	*C*	28th Oct. 1936—10 A.M.	5	10	18	Positive; type *C*.
Age of diseased tobacco (Source): 15—16 weeks.	2—14	*C*	28th Oct. and 1st Nov. 1936—10 A.M.	5—24	3—12	...	Negative.
Age of healthy tobacco infected: 6 7 weeks.	15	*C*	31st Oct. 1936—1 P.M.	24	8	22	Positive; type *C* & *D*.
	16	*C*	Do.	24	4	22	Positive; type *C*.
	17	*C*	Do.	24	7	12	Positive; type *X*.
	18—21	*C*	Do.	24	3—6	...	Negative.
	22	*C*	Do.	24	5	37	Positive; type *C*.
	23—24	*C*	Do.	24	4—8	...	Negative.
	25	*B*	28th Oct. 1936—10 A.M.	5	12	...	Do.
	26	*B*	31st Oct. 1936—1 P.M.	24	11	22	Positive; type *B*.

27—33	*B*	Do.	24	3—13	...	Negative.
34	*B*	1st Nov. 1936—3 P.M.	24	9	101	Positive; A type *X*
35	*B*	Do.	24	11	110	Positive; type *B*.
36	*B*	Do.	24	6	36	Positive; *A* type *B* or *C & D*.
37	*B*	Do.	24	8	...	Negative.
38	*B*	Do.	24	7	20	Positive; type *B*.
39	*B*	Do.	24	11	...	Negative.
40	*B*	Do.	24	9	20	Positive; type *B*.
41	*X* of *C*	31st Oct. 1936—1 P.M.	24	4	21	Positive; type *C & D*.
42	*X* of *C*	Do.	24	7	32	Positive; *C* type *X*.
43	*A* with a little of *C & D*.	Do.	24	7	...	Negative.
44	Do.	Do.	24	9	22	Positive; *C* type *X*.
45	Do.	1st Nov. 1936—3 P.M.	24	9	20	Positive; type *X*.
46	Do.	Do.	24	13	102	Positive; *C* type *X*.
47	Do.	Do.	24	7	...	Negative.
48	*A+B+C+D*	5th Nov. 1936—5 P.M.	21	26	140	Positive; type *X*.
49	Do.	Do.	21	28	...	Negative.
50	Do.	Do.	21	33	102	Positive; type *X*.
51	Do.	Do.	21	29	...	Negative. Experiment Nos. 48—51. The vectors were fed separately on diseased plants of individual types A, B, C & D and then transferred together to single healthy seedlings in each case. The result was a synthetic mixture *X*.

TABLE IV-A—*contd.*

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks
1	2	3	4	5	6	7	8
				(Hours)		(Days)	
Series v							
Time of experiment, 14th-16th December 1936	1—8	*A*	14th-16th Dec. 1936—10 A.M.—4 P.M.	6—24	3—16	...	Negative.
Age of diseased tobacco (Source): 16 weeks.	9—12	*B*	14th-15th Dec. 1936—10 A.M.—4-30 P.M.	6—7	5—11	...	Do.
Age of healthy tobacco infected: about 8 weeks.	13	*B*	15th Dec. 1936—9-30 A.M.	7	7	52	Positive: type *B*.
	14—15	*B*	15th-16th Dec. 1936—9-30 A.M.—4 P. M.	6—24	3—6	...	Negative.
	16	*B*	16th Dec. 1936—10 A.M.	6	7	68	Positive; type *B*.
	17—22	*C*	14th-16th Dec. 1936—10 A.M.—9-30 A.M.	7—24	6—13	...	Negative.
	23	*C*	16th Dec. 1936—10 A.M.	6	5	59	Positive; type *C*.
	24	*C*	Do.	6	8	55	Do.
	25—33	*D*	14th-16th Dec. 1936—10 A.M.—4 P.M.	6—7	4—14	...	Negative.
Series vi							
Time of experiment, 25th December, 1936-5th January 1937.	1	*A*	25th Dec. 1936—4 P.M.	18	5	94	Positive; type not clear.
	2	*A*	Do.	18	6	31	Positive; type *A*?

Age of diseased tobacco (Source) : 16—17 weeks.

Age of healthy tobacco infected : 6—8 weeks.

3—10	*A*	Do.	18	4—7	...	Negative.
11—18	*C*	Do.	18	4—9	...	Do.
19	*C*	Do.	18	6	47	Positive ; type *X*.
20	*C*	Do.	18	3	...	Negative.
21	*D*	26th Dec. 1936—10 A.M.	6	5	...	Do.
22	*D*	Do.	6	4	94	Positive ; type not clear.
23—30	*D*	Do.	6	4—9	...	Negative.
31	*A*+*B*+*C*+*D*	27th Dec. 1936—9 A.M.	6	11	...	Do.
32	*A*+*B*+*C*+*D*	Do.	6	13	52	Positive ; type *X*.
33	*A*+*B*+*C*+*D*	Do.	6	21	37	Do.
34	*A*+*B*+*C*+*D*	Do.	6	24	...	Negative.
35	*A*+*B*+*C*+*D*	Do.	6	18	93	Positive ; type *X*.
36—37	*A*+*B*+*C*+*D*	Do.	6	13—15	...	Negative.
38—39	*A* + *C*	Do.	6	11—15	...	Do.
40	*A* + *C*	Do.	6	13	36	Positive ; A type *X*.
41	*A* + *D*	Do.	6	7	37	Positive ; type *X*.
42	*A* + *D*	Do.	6	9	...	Negative.
43	*A* + *D*	Do.	6	11	47	Positive ; type *X*.
44—45	*B*	4th-5th Jan. 1937—2 P.M.	24	2—4	...	Negative.
46	*B*	Do.	24	6	87	Positive ; type not clear.
47—51	*B*	Do.	24	3—5	...	Negative.
52—56	*C*	Do.	24	3—5	...	Do.
57	*C*	Do.	24	6	31	Positive ; type *C*.
58—59	*C*	Do.	24	4	...	Negative.
60—64	*X*	4th Jan. 1937—2 P.M.	24	2—6	...	Do.
65	*X*	Do.	24	8	37	Positive ; type *X*.
66—67	*X*	Do.	24	4—6	...	Negative.

TABLE IV-A—*concld.*

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks
1	2	3	4	5	6	7	8
				(Hours)		(Days)	
Series vii							
Time of experiment, 6th-9th February 1937	1	*A*	6th Feb. 1937—3 P.M.	18	9	17	Positive ; type *X*.
	2—15	*A*	Do.	18	4—11	17	Negative.
Age of diseased tobacco (Source) : 22 weeks.	16—19	*B*	Do.	18	5—12	17	Do.
Age of healthy tobacco infected : about 7 weeks.	20	*B*	Do.	18	8	37	Positive ; type *B*.
	21—24	*B*	Do.	18	6—9	...	Negative.
	25	*B*	Do.	18	7	18	Positive ; type *B*.
	26—27	*B*	Do.	18	9	...	Negative.
	28	*B*	Do.	18	8	19	Positive ; type *B*.
	29	*B*	Do.	18	9	26	Do.
	30	*B*	Do.	18	9	...	Negative.
	31—45	*C*	Do.	18	5—13	...	Do.
	46—50	*D*	Do.	18	7—11	...	Do.

51	*D*	Do. .	18	7	20	Positive ; type not clear.
52	*D*	Do. .	18	6	24	Do.
53	*D*	Do. .	18	4	...	Negative.
54	*D*	Do. .	18	9	20	Positive ; type *X*
55	*D*	Do. .	18	6	...	Negative.
56	*D*	Do. .	18	6	22	Positive ; type not clear.
57 63	*D*	Do.	18	7—11	...	Negative.
64	*D*	Do. .	18	8	34	Positive ; type not clear.
65	*D*	Do. .	18	6	...	Negative.
66	*X*	7th Feb. 1937—2 P.M.	24	6	19	Positive ; type *X*.
67	*X*	Do. .	24	7	...	Negative.
68	*X*	Do. .	24	4	23	Positive ; type *X*.
69	*X*	Do. .	24	9	...	Negative.
70	*X*	Do. .	24	8	25	Positive ; type *X*.
71—73	*X*	Do. .	24	5—9	...	Negative.
74	*X*	Do. .	24	7	20	Positive ; type *X*.
75—77	*X*	Do. .	24	5—7	...	Negative
78—79	*X*	8th-9th Feb. 1937—2 P.M.	24	6—7	...	Do.
80	*X*	Do. .	24	5	11	Positive ; type *X*.

None of the controls of the various series developed leaf-curl disease.

TABLE IV-B

Particulars of Experiments performed to transmit the disease from tobacco to tobacco during 1937

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks
1	2		4	5	6	7	8
				(Hours)		(Days)	
Series i							
Time of experiment, 14th-15th October 1937	1	*D*	14th Oct. 1937—9 A.M.	7	4	34	Positive : type *X*.
Age of diseased tobacco (Source) : 13 weeks.	2	*D*	Do.	7	5	32	Do.
Age of healthy tocacco infected : about 9 weeks.	3—17	*D*	Do.	7—24	4—9	...	Negative.
	18—20	*X*	14th-15th Oct.1937—9 A.M.	24	4—6	...	Do.
Series ii							
Time of experiment, 5th-9th November 1937	1—2	*A*	5th Nov. 1937—4 P.M.	24	4—5	...	Do.
Age of diseased tobacco (Source) : 10 weeks.	3—6	*D*	Do.	18—24	5—10	...	Do.
Age of healthy tobacco infected : 8 weeks.	7—8	*X*	Do.	18—24	1—8	...	Do.
	9—10	*A* type *X*	Do.	18—24	5—7	...	Do.
	11—15	*A* & *C*	Do.	24	1—9	...	Do.

	16 18	*A* & *C*	6th November 1937—2-30 P.M.	17½	5 10	...	Do.
	19—21	*B*	Do.	17½	2—5	...	Do.
	22	*C*	Do.	17½	6	30	Positive; type trace of C. recovered.
	23—30	*C*	Do.	17½	2—10	...	Negative.
	31	*C*	8th-9th November 1937—11-15 A.M.	24	12	25	Positive; *C* type *X*.
	32	*C*	Do.	24	3	...	Negative.
	33 35	*B*	Do.	24	2—5	...	Do.
Series iii.							
Time of experiment, 29th November-1st December 1937	1—6	*C*	29th November 1937—NOON.	24	2—8	...	Do.
Age of diseased tobacco (Source): 13 weeks.	7—9	*B*	Do.	24	1—5		Do.
Age of healthy tobacco infected: 7 weeks.	10—13	*A*	Do.	24	2—4	...	Do.
	14 15	*X*	Do.	24	2—3	...	Do.
	16—21	*D*	29th November 1937—4 P.M.	24	1—5	...	Do.
	22—23	*X*	30th November-1st December 1937—4 P.M.	24	8—9	...	Do.
	24—25	*C*	Do.	24	5—6	...	Do.

None of the controls of the various series developed leaf-curl disease.

TABLE V

Particulars of Experiments performed to transmit the disease from Ageratum conyzoides *to tobacco during 1936 and 1937*

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks
1	2	3	4	5	6	7	8
				(Hours)		(Days)	
Series i							
Time of experiment, 16th-17th December 1936	1	...	16th-17th. December 1936—3 P.M.	25	16	34	Positive; type *D*.
Age of diseased *Ageratum* (Source): not known, obtained from field.	2	...	Do.	25	6	33	Do.
Age of healthy tobacco infected, 9 weeks.	3—6	...	Do.	25	6—11	...	Negative.
Time of experiment, 5th-8th September 1937	1	...	5th Sept. 1937—2 P.M.	25	3	55	Positive; type *D*.
Age of diseased *Ageratum* (Source): not known, obtained from field.	2—6	...	Do.	25	2—4	...	Negative.
Age of healthy tocacco infected, 8 weeks.	7	...	6th Sept. 1937—11 A.M.	21	4	...	Do.
	8	...	Do.	21	9	16	Positive; type *D*.
	9—12	...	Do.	21	3—5	...	Negative.
	13	...	Do.	21	7	54	Positive; type *D*.
	14	...	Do.	21	4	54	Do.
	15	...	Do.	21	3	15	Do.
	16	...	Do.	21	7	25	Do.
	17—19	...	Do.	21	5—7	...	Negative.
	20	...	Do.	21	3	49	Positive; type *D*.
	21	...	7th Sept. 1937—5 P.M.	16	7	24	Do.

		...	Do.	16	8	...	Negative.
	23	...	Do.	16	10	53	Positive ; type *D*
	24	...	Do.	16	14	...	Negative.
	25	...	7th-8th Sept. 1937—5 P.M.	16	13	15	Positive ; type *D*
	26	...	Do.	16	5	24	Do.
	27	...	Do.	16	14	14	Do.
	28	...	Do.	16	16	66	Do.
	29	...	Do.	16	8	13	Do.
	30	...	Do.	16	11	83	Do.
	31	...	Do.	16	12	...	Negative.
	32	...	Do.	16	8	24	Positive; type *D*.
	33	...	Do.	16	5	66	Positive ; *D* type *X*.
	34	...	Do.	16	12	53	Positive ; type *D*.
	35	...	Do.	16	10	35	Do.
	36	...	Do.	16	7	24	Positive ; type *D* ? (Recovered).
	37	...	Do.	16	9	65	Positive : type *D*.
	38	...	Do.	16	7	68	Do.
	39	...	Do.	16	7	64	Do.
	40	...	Do.	16	9	66	Do.
Series ii							
Time of experiment, 26-28th September 1937 Age of diseased *Ageratum* (Source) : not known, obtained from field. Age of healthy tobacco infected : 6 weeks.	1—3	...	26th Sept. 1937—10 A.M.	25	7—11	...	Negative.
	4	...	Do.	25	6	15	Positive ; type *D*.
	5	...	Do.	25	11	15	Do.
	6—7	...	Do.	25	7—8	...	Negative.
	8	...	Do.	25	9	23	Positive ; type *D*.
	9—10	...	Do.	25	6—8	...	Negative.
	11	...	Do.	25	12	30	Positive ; type *D*.
	12—15	...	Do.	25	8—13	...	Negative.
	16	...	Do.	25	9	15	Positive ; type *D*.

TABLE V—*contd.*

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks
1	2	3	4	5	6	7	8
				(Hours)		(Days)	
Series ii—contd.							
	17	...	26th Sept. 1937—10 A.M.	25	11	...	Negative.
	18	...	Do.	25	6	27	Positive ; type *D*.
	19—20	...	Do.	25	7—11	...	Negative.
	21	...	27th-28th Sept. 1937—11 A.M.—7-30 A.M.	20½	10	24	Positive ; type *D*.
	22—24	...	Do.	20½	13—21	...	Negative.
	25	...	Do.	20½	24	11	Positive ; *D* type *X*.
	26—30	...	Do.	20½	14—26	...	Negative.
Series iii'							
Time of experiment, 2nd-5th November 1937.	1	...	2nd Nov. 1937—4 P.M.	44	6	24	Positive ; type *D*.
	2—7	...	Do.	44	5—8	...	Negative.
Age of diseased *Ageratum* (Source) : 13 weeks.	8—14	...	4th-5th Nov. 1937.—NOON.	29	6—12	...	Do.
Age of healthy tobacco infected : 7 weeks.	15	...	Do.	29	10	33	Positive ; type *D*.
	16—17	...	Do.	29	6—8	...	Negative.

	18	...	Do. .	29	9	31	Positive ; type *D*.
	19	...	Do. .	24	9	...	Negative.
	20	...	Do. .	24	13	27	Positive ; type *D*.
Series iv							
Time of experiment, 22nd-26th November 1937.	1—2	...	22nd Nov. 1937—NOON.	24	4—7	...	Negative.
Age of diseased *Ageratum* (Source) : 16 weeks.	3	...	Do. .	24	8	41	Positive ; type *D*.
Age of healthy tobacco infected : 6 weeks.	4		Do. .	24	1	19	Do.
	5	...	Do. .	24	3	32	Positive ; type *D* (Recovered).
	6	...	Do. .	24	5	19	Positive ; type *D*.
	7	...	Do. .	24	1	19	Do.
	8	...	Do. .	24	5	30	Do.
	9	...	Do. .	24	5	19	Do.
	10	...	22nd Nov. 1937—4 P.M.	24	1	...	Negative.
	11	...	Do. .	24	5	22	Positive ; type *D* ? (Recovered).
	12	...	Do. .	24	6	19	Positive ; type *D*.
	13	...	Do. .	24	4	19	Do.
	14	...	23rd Nov. 1937—4 P.M.	24	2	...	Negative.
	15	...	Do. .	24	4	49	Positive ; type *D*.
	16—18	...	Do. .	24	4—7	...	Negative.
	19	...	Do. .	24	4	26	Positive : type *D*.
	20	...	Do. .	24	5	28	Do.
	21	...	24th—26th Nov. 1937—3 P.M.—9 A.M.	42	3	...	Negative.
	22	...	Do. .	42	4	26	Positive ; type *D*.
	23—30	...	Do. .	42	2—6	...	Negative.

None of the controls of the various series developed leaf-curl disease.

TABLE VI

Particulars of Experiments performed to transmit the disease from tobacco to Ageratum conyzoides *during 1937*

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks
1	2	3	4	5	6	7	8
				(Hours)		(Days)	
Series i							
Time of experiment, 29th-30th October 1937.	1—9	D	29th-30th Oct. 1937—5 P.M.—9 A.M.	16	2—9	15—47 average.	Positive; type resembled that occurred in nature. Profuse branching resulted.
Age of diseased tobacco (Source): 9 weeks.	...	...	...	...	...	35	
Age of healthy *Ageratum* infected: 11 weeks.							

None of the controls of the series developed leaf-curl disease.

TABLE VII

Particulars of Experiments performed to transmit the disease from Ageratum conyzoides *to sann-hemp during 1937*

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks
1	2	3	4	5	6	7	8
				(Hours)		(Days)	
Series i							
Time of experiment, 2nd-5th Sept. 1937.	1	...	3rd Sept. 1937—11 A.M.	23	3	78	Positive; type S. V. 2 Side shoots also diseased.
Age of diseased *Ageratum* (Source): 11 weeks.	2—5	...	2nd-4th Sept. 1937—NOON—10 A.M.	20—23	2	...	Negative.
Age of healthy sann-hemp infected: 9 weeks.	6—13	...	3rd-5th Sept. 1937—4 P.M.—10 A.M.	18—23	2—7	...	Do.
	14	...	4th Sept. 1937—10 A.M.	18	5	72	Positive; type S. V. 2.
	15—18	...	Do.	18	2—4	...	Negative.

None of the controls of this series developed leaf-curl disease.

TABLE VIII

Particulars of Experiments performed to transmit the disease from sann-hemp to Ageratum conyzoides *during 1937*

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks
1	2	3	4	5	6	7	8
				(Hours)		(Days)	
Series i							
Time of experiment, 11th-12th Oct. 1937.	1	S. V. 1.	11th-12th Oct. 1937—3 P.M.	24	9	...	Negative.
Age of diseased sann-hemp (Source): 11 weeks.	2—10	S. V. 1	Do.	24	5—12	49—69 average. 59	Positive; type resembled that occurred in nature.
Age of healthy *Ageratum* infected : 11 weeks.	...	...	...	...	...	...	Profuse branching resulted.
Series ii							
	1—11	S. V. 2	11th-12th Oct. 1937—3 P.M.	24	5—11	38—100 Average. 63	Positive; type resembled that occurred in nature. Profuse branching resulted.

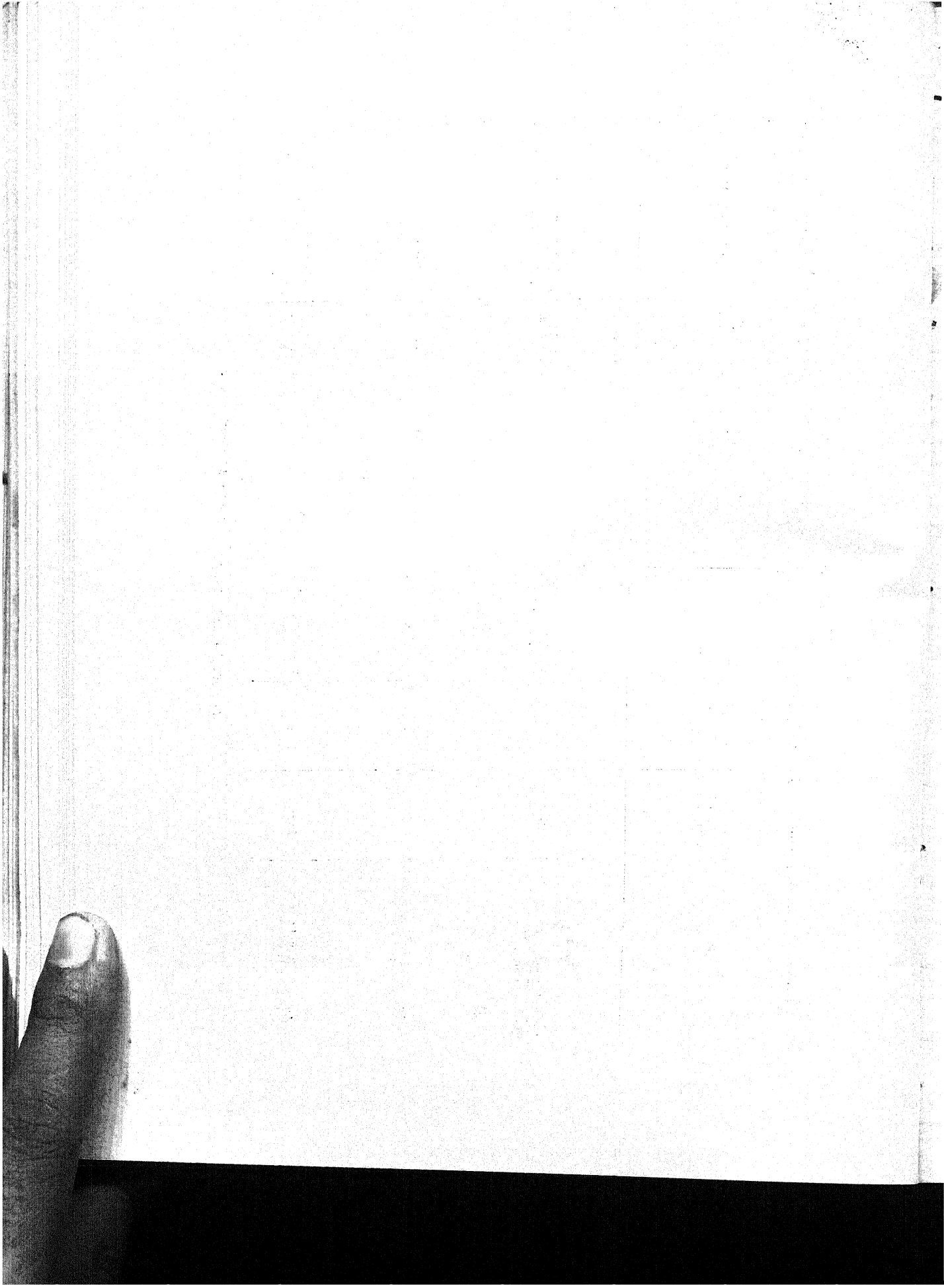
None of the controls of this series developed leaf-curl disease.

TABLE IX

Particulars of Experiments performed to transmit the disease from Ageratum conyzoides *to* Ageratum Conyzoides *during 1937*

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks
1	2	3	4	5	6	7	8
				(Hours)		(Days)	
Series i.							
Time of experiment, 10th-11th October 1937 and 29th-31st October 1937.	1—19	...	10th-11th Oct. 1937—11 A.M.	24	5—11	13—71 average 42.	Positive; type resembled that occurred in nature. Profuse branching resulted.
Age of diseased *Ageratum* (Source): 14 weeks.	20	...	Do.	24	9	...	Negative.
Age of healthy *Ageratum* infected: 11 weeks.	21—24	...	29th-31st Oct. 1937—5 P.M.	22—24	6—10	27—39 average 34.	Positive; type resembled that occurred in nature Profuse branching resulted.

None of the controls of this series developed leaf-curl disease.



THE PHYTOPHAGOUS CHALCIDOID, *EURYTOMA SAMSONOVI* VAS., A SERIOUS PEST OF APRICOT AND SOME OTHER FRUITS IN INDIA

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(With Plate XI)

Introduction

Chalcidoidea are well known as important parasites of insect pests. The phytophagous or plant feeding habits of certain families of this group, although seriously doubted at one time, are now fully established and numerous species are reported to be serious pests of some important cereal crops and fruits in various parts of the world. The notorious jointworm of wheat, *Harmolita tritici* (Fitch.) is widely distributed in the United States of America and several European countries, while *Eurytoma amygdali* End. infests almond, plum and prunes in Cyprus, Syria, Palestine, Italy, Russia, etc. The whole family Agaonidae on account of their food plants are known as fig insects, while a large number of Torymidae and Eurytomidae infest seeds, grains and several cultivated grasses [Crossby, 1909]. Gahan [1922] listed all the phytophagous Chalcidoidea excepting fig insects, and the species enumerated by him belong to as many as six families. Since 1922 a large number of new phytophagous forms have been discovered in several parts of the world.

The species included under the Chalcidoid families referred to above have a variety of feeding habits. Some genera like *Eurytoma*, *Tetrastichus*, etc., include both parasitic and phytophagous species [Phillips, 1927 ; Ishii, 1931]. There are some species which in the early stages of their development attack other insects and are thus parasitic, and complete the rest of their life-cycles on plant tissues [Phillips, 1927 ; Puzanov-Malyschev, 1936].

In India, besides the fig insects, only two phytophagous Chalcidoids are known to exist, *viz.*, *Eurytoma samsonovi* Vasiljev, which attacks apricot, peach, etc., in the North-West Frontier Province, and *Bruchophagus mellipes*

*The work of Mr. Batra incorporated in this paper was done by him when he was a post-graduate student in Entomology at the Imperia Agricultural Research Institute,

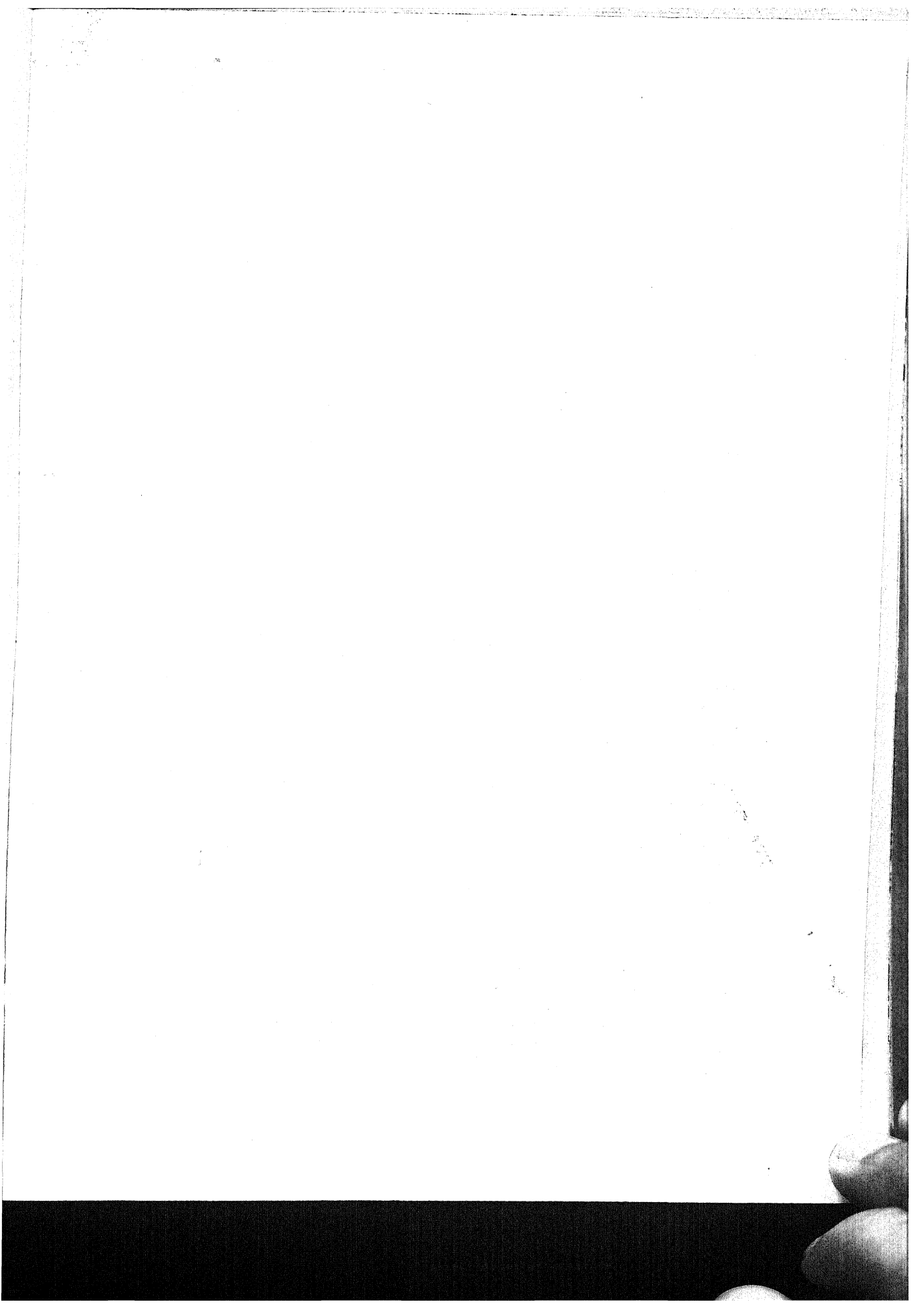
Gohar, feeding on *agathi* and *daincha* (*Sesbania aegyptica*) in Bihar and South India. Of these, *E. samsonovi* is the most important and the history of its discovery is rather long and interesting and is described below :—

Discovery of *Eurytoma samsonovi* in India and a review of allied pests in other parts of the world

In May 1910, Mr. Robertson Brown, Agricultural Officer in the North-West Frontier Province, sent a consignment of infested green apricots to the Imperial Entomologist for the identification of the pest concerned and suggestions for its control. Soon after the pest larvae were taken out of the fruits in the Insectary at Pusa, they died and at the request of the Imperial Entomologist another consignment was received in 1911. The material was carefully reared and a number of adult Chalcidoid wasps emerged between the 25th March and the middle of April. The specimens belonged to the family Eurytomidae, but as very few phytophagous Eurytomids were known at that time, they were taken to be the parasites of the real pest, and search for ' the pest ' continued. Infested fruits were kept under observation at Pusa during 1915-16, and again in 1926-27, but nothing except the Eurytomid emerged. This and the fact that by that time several Eurytomid pests had been discovered in other parts of the world gave rise to the idea that perhaps the so-called parasite was the real pest and therefore the Imperial Entomologist sent one of his assistants to the North-West Frontier Province to investigate the biology of the Chalcidoid at the spot. As a result of this investigation it was found that the Eurytomid itself was the real pest and the specimens were subsequently identified through the courtesy of Mr. Howard of the United States of America as *Eurytoma samsonovi* Vas. This species was originally described from Central Asia in 1915 where it is a serious pest of apricots, some plums and prunes, etc. [Vasiljev, 1915].

During 1928 the pest was studied in some detail in the Insectary at Pusa and a brief account of the work was published in the Report of the Imperial Entomologist for 1928-29 [Fletcher, 1930]. In 1934 when the junior author (H. N. B.) was working as a post-graduate student at Pusa, he visited the North-West Frontier Province and took observations on this pest in the field. In 1937 the Imperial Entomologist himself studied the pest in the field and in the spring of 1938 sent his assistant, Mr. H. L. Bhatia, to make observations on some points that were still obscure. The results of the studies carried out during different periods since 1910 are summarised in the following pages.

Before describing *Eurytoma samsonovi*, a brief account of the pests belonging to genus *Eurytoma* and to the other Indian genus, *Bruchophagus*, which occur in other countries may be given. Several species of *Eurytoma* occur in different parts of the world and attack several kinds of fruits, seeds, stems of plants, etc. Their life and seasonal histories in general are almost similar. The more important pests are listed below ;—



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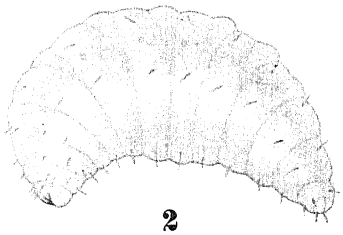

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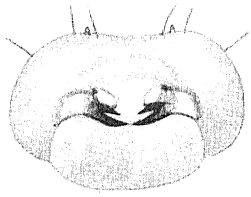

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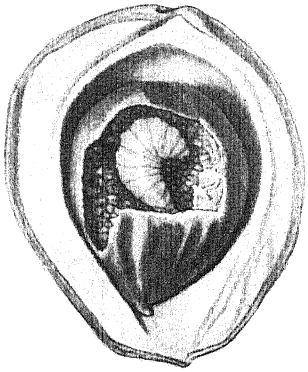

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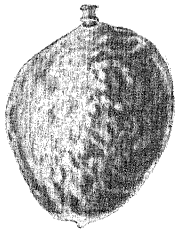

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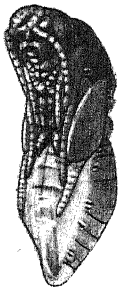

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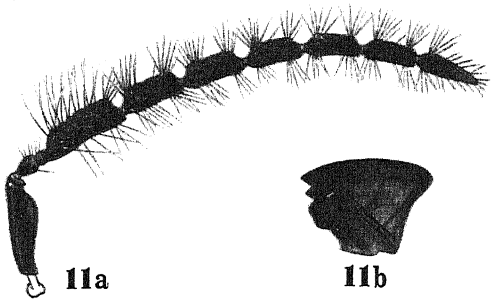

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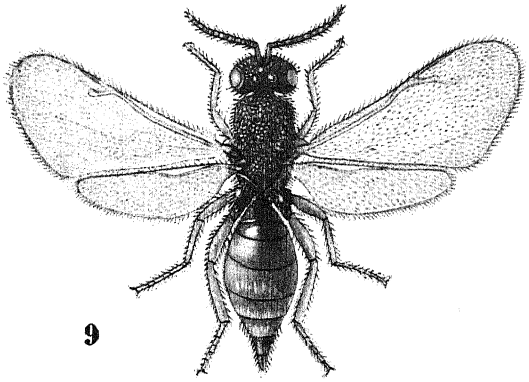

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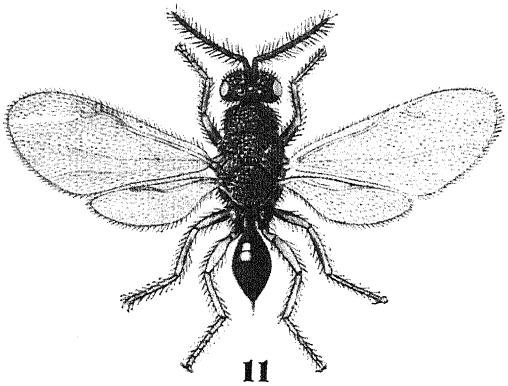

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PLATE XI

Eurytoma samsonori Vas.

FIG. 1. Young grub 4-5 days old (×6)

2. Full-grown grub (×6)

3. Mouth parts of the same, note the powerful mandibles (×32)

4. Section of apricot fruit, showing the seed, the kernel of which has been eaten out by the grub but whose covering (testa) is still intact (×1)

5. Same, with the testa ruptured, showing the grub inside (×2)

6. Infested apricot fruit (×1)

7. Same, out of which the adult Chalcidoid has emerged by boring a hole (×1)

8. & 9. Pupa and adult female (×6)

10 & 11. Pupa and adult male (×6)

11*a-b*. Antenna and mandible of the male (×40)

PLATE XI

Bruchophora sansonori Vas.

Fig. 1. Young grub 4-5 days old (×6)
2. Full-grown grub (×6)
3. Mouth parts of the same, note the powerful mandibles (×32)
4. Section of apricot fruit, showing the seed, the kernel of which has been eaten out by the grub but whose covering (testa) is still intact (×1)
5. Same, with the testa ruptured, showing the grub inside (×2)
6. Infested apricot fruit (×1)
7. Same, out of which the adult Chalcidoid has emerged by boring a hole (×1)
8 & 9. Pupa and adult female (×6)
10 & 11. Pupa and adult male (×6)
11a-b. Antenna and mandible of the male (×40)

Eurytoma amygdali End.—A very serious pest of almonds in Cyprus [Morris, 1930], Syria, Palestine [Aharoni, 1916], Bulgaria, Italy, and also a pest of apricots, plums and prunes in Russia [Puzanov-Malyschev, 1930; Vodinskaya, 1932].

E. orchidearum Westw.—A pest of orchids (*Cattleya* spp.) in Holland [Van Poeteren, 1932].

E. plotnikovi Niks.—Attacks *Pistacia* spp. in Russia [Nikol'skaya, 1935].

E. fellis Gir.—Causes galls in citrus stems in Queensland, New South Wales [Noble, 1933].

E. onobrychidis Niks.—Infests seeds of *Onobrychis sativa* in Russia [Nikol'skaya, 1933].

Bruchophagus gibbus Boh.—Infests clover seeds in Europe and America [Sorenson, 1935], lucern and alfalfa seeds in Russia [Ponomarenko, 1934; Nikol'skaya, 1932].

SEASONAL HISTORY AND NATURE OF DAMAGE

The adult Chalcidoids appear in the orchards in the plains of the North-West Frontier Province during the second half of March, while at higher localities such as Parachinar their emergence is delayed up to the middle of April. At Haripur, in 1938, it made its appearance about the 20th March when the apricot fruits were yet of the size of small almonds. March 1938 was unusually wet and cold in that area and no copulating individuals were observed till the end of the month. Copulation generally took place while the individuals were flying in the air.

The exact method of oviposition has not yet been observed but it appears that the eggs are laid on the endocarp (stone) or inside the young seeds which is still tender. The female pierces the fruit with the help of its ovipositor. The young grubs on hatching work their way in and feed on the soft kernel of the seed. A parcel of raw apricots received from Haripur about the 20th April contained young grubs, 4 to 5 days old, feeding on the seed kernel. The young grub is translucent-white in colour and has a soft body but strong mouth parts (Plate XI, fig. 1). It voraciously feeds on the kernel leaving its coat in tact, which consequently presents a shrivelled and papery appearance (Plate XI, figs. 4 and 5). On account of the young seed having mostly been eaten away, the infested fruit looses its fresh green colour, becomes dark grey, shrivels up, and with even moderately strong breeze falls off the tree (Plate XI, fig. 6). Of about 1,200 fruits picked up from under the trees at Haripur during May 1938, about 85 per cent contained young grubs.

The fallen fruits get hardened and in many cases their pulps decompose, making the stones bare but the grubs continue to feed on the kernel inside the stones throughout the summer and are full grown in September and October. The fully fed grub (Plate XI, fig. 2) is very plump and fleshy and lies

curved up inside the seed. On the onset of winter the grubs go into hibernation and remain in this condition up to about the end of February when pupation takes place. The pupal period is two to three weeks and is also passed inside the seed. About the middle of March the adults emerge and leave the dry fruit by gnawing a clean hole through it (Plate XI, fig. 7). The cycle as outlined above is then repeated.

From the foregoing it will be noticed that the grub (larval) stage lasts for nine or ten months out of about one year in which a life-cycle is completed. Sometimes the grubs do not pupate next spring but pass the second summer and winter in the same stage. This happened at Pusa when the pest was being studied during 1916-17. In May 1937 a collection of about 450 infested fruits was obtained from Peshawar and Haripur and was kept in the Insectary at New Delhi. In the spring of 1938 while adults successfully emerged out of about 40 per cent and the grubs inside about the same number were found dead, in the remaining 20 per cent the grubs did not pupate and were still alive in August 1938. Probably they will pupate next spring. Thus, while normally a life-cycle takes about one year for completion, sometimes it extends over two years, most of the period being passed in the grub stage.

The seasonal history described above is roughly similar in broad outline to those of other Eurytomid pests which occur in Europe and America, though of course the actual times of the appearance of the adults in orchards and of hibernation of the grubs depend on the climate and weather conditions prevailing in different countries [Phillips and Poos, 1921 ; James, 1927 ; Puzanov-Malyschev, 1930 and 1936 ; etc.].

Description of various stages in the life-history

As already stated, the eggs of *Eurytoma samsonovi* have not yet been observed. The young grub (Plate XI, fig. 1), about four or five days old, is about 2·5 mm. long. It is thick in the middle and tapering towards the two ends of the body, the breadth in the thickest part being 0·75 mm. It is greyish in colour but the mouth parts are brown on account of heavier chitinization. The body is almost transparent, therefore, the course of the alimentary canal with its coloured contents can be easily made out. The grub invariably remains in a curved position, is legless and its head is always retracted inside the prothoracic region. The full-fed grub (Plate XI, fig. 2) is very large and plump, measuring about 7·5 × 3·3 mm., otherwise it does not differ in appearance from the young grub. Nine pairs of spiracles which are brown in colour and circular in outline, can be easily made out in the lateral sides of its body, which have also four to five rows of small spines or setae in the thoracic region and three to four rows in the region of the abdomen.

The pupae are dimorphic, the female pupa measuring 6·5 × 2·5 mm., being brown in the abdominal region and the male being smaller (5·5 × 2mm.) having almost a dark body (Plate XI, figs. 8 and 10). However, freshly formed pupae of both sexes are much brighter in colour. The adults

(Plate XI, figs. 9 and 11) like the pupae are dimorphic. The female is bigger ($7 \cdot 0 \times 1 \cdot 75$ mm.) and much brighter in colour than the male, which measures about $5 \times 1 \cdot 5$ mm. The eyes in both sexes are of beautiful red colour and the bodies are finely pubescent. The abdomen of the male is proportionately much smaller as compared to the thorax than it is in the female. The ocelli are almost in a straight line in the male, whereas they form a triangle in the case of the female. The antennae are geniculate as is usual in the Chalcidoidea, but are very different in the two sexes. The structure of the male antenna is almost similar to that of *Harmolita graminicola* Gir., described by James [1926]. Its scape (Plate XI, fig. 11*a*) is shorter and stouter than in the female. The funiculus consists of eight segments exclusive of a small ring-like joint between the first and second segments. The whole length of the funiculus is covered with sensony pits and setae or sensilli, the latter being longer and more numerous in the male than in the female. The mandibles (Plate XI, fig. 11*b*) are well developed, subtriangular in outline. Each mandible has a pair of sharp teeth close to which there is a pad-like chitinous ridge which has a crushing function.

Distribution, food plants and economic status

The most favourite host of *Eurytoma samsonovi* is apricot fruit. Peach and plum fruits are also attacked to some extent. The pest is widely distributed in the North-West Frontier Province including the hilly areas, *e.g.*, Parachinar, etc., but is serious in the plains only. It is especially bad in the Hazara district, the home of apricot fruits. In this locality, according to the report of the Director of Agriculture, as much as 70 per cent of the fruit gets attacked and falls off the trees. In the Haripur tehsil alone about 400 acres are under apricot orchards, containing about 70,000 trees. The average yield per tree is about two maunds, fetching about Rs. 5 per maund. The loss, even if only 50 per cent fruits fall off, works out at about four lakhs of rupees per annum. The extent of loss caused by the pest in the whole of the Province can therefore be easily imagined.

Several varieties of apricots are cultivated in the North-West Frontier Province and all of them are not equally susceptible to the attack of this pest. The *Kouri* variety which is the earliest to mature and fruits most profusely is most heavily attacked. Fortunately this variety is not highly priced. The latest variety, *jabhan*, which is the most delicious and sells at about Rs. 10 per maund suffers the least, about 10-20 per cent of the fruits being only affected. The other varieties, *bouei*, *sufeda* and *lallan* are intermediate in value as well as in the loss sustained.

It is of interest to record that one of us (H. S. P.) looked for this pest in the apricot orchards of Baluchistan in the summer of 1937, but found all localities free from it. There is also no report of the pest on any fruit in Kashmir, therefore its only habitat known at present is the North-West Frontier Province.

Control measures

In view of the nature of the life-history which indicates that the pest remains in grub stage inside the fallen fruits for about nine months in the year, the Imperial Entomologist suggested to the Agricultural Officer of the North-West Frontier Province that he should get all the fallen fruit collected and destroyed in some orchards and watch the incidence of the pest in the succeeding years. This method which does not involve much expenditure was tried by Mr. Robertson Brown in 1930 and found very effective.

After Mr. Robertson Brown's retirement, the control measure was tested on a large scale under the direction of Col. Noel, who stated in a letter to the Imperial Entomologist in 1935 " that the campaign had resulted in a very considerable reduction of the pest." We understand that a fruit grower in the Haripur tehsil who had got only 45 mds. of fruit from his orchard in 1932, but had collected and destroyed all the fallen fruit during the summer of that year, got as much as 135 mds. from the same orchard next year.

In 1937, when the senior author visited Peshawar and met Col. Noel, the latter again spoke of the efficacy of the method and told him that he was moving his Government to introduce legislation to make it obligatory for owners of orchards to collect and destroy all fallen fruit under penalty of a fine.

It is interesting to record that in the case of Eurytomid pests in other countries also, *e.g.*, Cyprus, Russia, Queensland, etc., control measures almost similar to those described above are being advocated [Morris, 1930 ; Vodinskaya, 1932 ; Noble, 1933 ; etc.].

As regards natural enemies, Nikol'skaya [1932, 1933, 1935] has recorded several parasites of Eurytomid pests (*Eurytoma* spp., and *Bruchophagus gibbus*) in Russia, and Phillips and Poos [1921] have done the same in the case of the joint-worm of wheat in the United States of America, but so far there is no record of any parasite of *Eurytoma samsonovi* from India.

Summary

1. Several species of phytophagous Chalcidoidea occur as pests of wheat, figs, clover seeds, almonds, pistachio, orchids, etc., in various parts of the world.

2. *Eurytoma samsonovi* Vasiljev, which attacks apricot, peach, etc., in the North-West Frontier Province, was first bred out from infested apricots in 1911, but it was not till about sixteen years later that its exact status as a pest of apricot and other fruits in the province was determined.

3. Observations on the biology of this insect were made in the North-West Frontier Province, from time to time, since 1928. In the plains, the adults appear early in spring, copulate and the females oviposit inside the young seeds of apricot and other fruits. The grubs feed throughout the summer and hibernate in winter at the end of which pupation takes place. A proportion of the grubs fail to pupate and continue as grubs through the

whole of the next summer and winter, prolonging the life-cycle to two or three years. Short descriptions of the young and full-fed larvae, pupa and adult are given.

4. Apricot fruit is the most favourite host of *E. samsonovi* which though also found in the hilly areas of the North-West Frontier Province is serious pest only in the plains.

5. On the recommendation of the Imperial Entomologist, collecting and burning of all fallen fruit in the orchards was tried on a large scale as a method of control, in the North-West Frontier Province, and found very effective.

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*STUDIES IN THE PERIODIC PARTIAL FAILURES OF AMERICAN COTTON IN THE PUNJAB

I. EARLY BREAKDOWN OF THE PHOTOSYNTHETIC APPARATUS

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(With Plates XII-XIV)

Introduction

In a preliminary note an early breakdown of the chloroplasts in the green leaves was described as an abnormal feature of the American cotton plants in the Punjab [Dastur, 1936]. At that time the different stages of the disintegrating process were not traced and the causes that give rise to the early breakdown of the chloroplasts were not investigated. This abnormal feature of apparently healthy leaves of the 4F cotton plants has now been studied during the cotton seasons of 1936 and 1937 and the important facts which have been discovered are summarised below :—

Chloroplasts are known to be stable protoplasmic bodies, which normally do not undergo disintegration in the functioning leaves, except under special conditions. The disintegration of the chloroplasts is reported to occur in plants suffering from bacterial [Smith, 1924] or virus diseases [Sorokin, 1927 ; Eckerson, 1926] and in plants attacked with insects [Painter, 1930]. No instance is recorded where the chloroplasts are found to disintegrate on account of some physiological disturbance in the plant. It is possible that such breakdown of the chloroplasts, as is found in the cotton plant, occurs in other plants as well and has merely escaped notice of the investigators.

Methods

The methods adopted to trace the different stages of disintegration were simple. Leaves of different ages were collected at regular intervals during the cotton season and were fixed in Flemming's weaker solution or Farmer's fluid

* This work was done in the Punjab Physiological (Cotton Failure) Scheme submitted by the Punjab Government and financed by the Indian Central Cotton Committee, Bombay.

or 4 per cent formalin. Sections of leaves were cut mostly by hand but sometimes by microtome. It is necessary to fix the leaves as an examination of unfixed material will lead to erroneous conclusions. Of various stains tried, haematoxylin and methyl violet gave the best results. Amongst the combination stains the Stoughton method was found most satisfactory. Though many varieties of exotic and indigenous cotton plants were examined, the following facts relate only to the 4F (*G. hirsutum*) cotton plant.

Investigation

The breakdown of chloroplasts and consequently of the photosynthetic system is found to occur in two ways, arising from two different causes. The two types of breakdown are classed as (A) mechanical and (B) chemical, and they are separately dealt with.

A. Mechanical breakdown of the chloroplasts

This type of breakdown of the chloroplasts is always preceded by a gradual accumulation of starch inside them. Normally starch does not accumulate inside the chloroplasts, as the starch that is stored up in them during the day is dissolved and removed at night. The same normal process is found to occur in the leaves of the cotton plant during the early part of the season. Towards the end of July and later leaves fully packed with starch begin to appear. The leaves collected at dawn are found to be full of starch. This indicates that dissolution and the removal of starch does not occur at night, though it continues to be formed and stored up in the chloroplasts during the day. The starch grains are found to grow bigger and thus increase in size on account of the fresh deposition of starch layers during the day.

A microscopic examination of such a leaf shows the distended palisade and spongy cells packed fully with unusually big starch grains which are joined laterally with one another, leaving no interspaces between them (Plate XII, figs. 3, 4, 5 ; Plate XIV, fig. 1). The chloroplasts in the cells are no longer distinguishable and are reduced to thin films surrounding the starch grains. The green colouring matter of the chloroplasts has come out in the form of small globules and covers the starch grains. The chloroplasts have bulged out and fused with one another. The palisade cells present the appearance of a reticulate network with starch grains occupying the meshes of the network and the bulged chloroplasts forming the net (Plate XII, figs. 3, 4). At many points the network of the chloroplasts is found broken due to the pressure of the starch grains. The starch grains in some cases get dissolved, leaving the broken fragments of the chloroplastic network in the cells. All such

stages of mechanical breakdown of the chloroplasts can be seen by suitable staining. The starch grains remain unstained while the chloroplasts are stained. Substances giving the reactions of tannins appear in the cell after the accumulation of starch has occurred (Plate XII, fig. 3 ; Plate XIV, fig. 1). The tannins first make their appearance on the lines of fusion of the starch grains and later cover them completely. The starch grains are so completely enveloped by tannins that starch-iodine reaction is not given by them, but the grains can still be distinguished by their size and shape. If the tannins and their derivatives are removed by suitable solvents the starch-iodine reaction is obtained at once. A palisade or a spongy cell may thus contain broken bits of chloroplasts, globules of green colouring matter, starch and tannins. The non-hydrolysis of starch very probably caused by some interference in the enzymic activities of the cell is thus responsible for the breakdown of the photosynthetic apparatus. Photosynthetic activity begins to decrease as starch remains unhydrolised and accumulates in the chloroplasts, and finally it comes to a standstill. The leaf continues to appear green and healthy so long as the globules of chlorophyll that have come out of the plastids remain undecomposed. Many such leaves with their load of starch and tannin or tannin alone are shed ultimately. In many cases the anthocyanin pigments develop in the epidermis prior to shedding.

B. Chemical breakdown of the chloroplasts

The second type of disintegration of chloroplasts is found to occur in young and half-matured leaves. The chloroplasts in the leaves of the cotton plant are round bodies which are stained uniformly by various dyes (Plate XII, fig. 1 ; Plate XIV, fig. 4). The first symptom of disintegration is the appearance of minute cavities in the chloroplasts (Plate XII, fig. 2 ; Plate XIV, fig. 4). In this type of breakdown the chloroplasts show swelling and vacuolation without starch accumulation. By further swelling of the chloroplasts the cavities enlarge and adjoining chloroplasts fuse together. They no longer appear as distinct bodies but become a sponge-like mass, filling the whole cell (Plate XII, fig. 2, Plate XIII, figs. 3, 4 ; Plate XIV, fig. 2). In the case of 4F Punjab-American cotton this type of disintegration can be seen with difficulty as the swelling is not very pronounced but in the case of Egyptian and Cambodia cotton plants all the stages of the process can be seen clearly (Plate XIII, figs. 3, 4). The swelling and ultimate dissolution of the chloroplasts is also accompanied by the formation of tannins in the cells (Plate XIII, fig. 4 ; Plate XIV, fig. 2). The chloroplasts become covered with tannins and can still be distinguished by the circular cavities.

In some leaves of the 4F and other cotton plants, small granules, one to three in number, appear in the body of the chloroplasts prior to the formation of circular cavities (Plate XIII, figs. 1, 2 ; Plate XIV, fig. 4). These granules are stained by methyl violet and other dyes in properly fixed materials. When they come out of the chloroplasts they leave cavities in them similar to those described above. They can be mistaken for spores which they are not. In some cells these granules are very minute, while in other cases they are larger and could be spotted easily.

Zirkle [1926], in an extensive study of the chloroplasts in higher plants, has described the presence of two types of granules in them. He has shown that the granules of the smaller type are not a part of the chromatophores, as reported by previous workers, while the larger granules are small pores or vacuoles in the chloroplasts. The granules observed in the chloroplasts of the cotton leaves would seem to be of a different type from those described by Zirkle. They appear to be distinct bodies which leave definite cavities in the plastids when they come out.

This type of breakdown of the chloroplasts develops first in the half-matured leaves and then spreads to the young leaves. The swollen sponge-like mass of the chloroplasts and tannins is the chief feature of the second type of breakdown of the photosynthetic apparatus. Both these types of disintegration may occur in the leaves of the same plant. The time when the disintegration sets in varies from plant to plant and particularly from field to field. It develops early in plants that later show the external symptoms of the ' disease ', while such premature breakdown of the chloroplasts does not occur in normal plants. These relationships between the breakdown of the photosynthetic apparatus and the bad opening of the bolls can be seen best in an extreme case of bad opening of the bolls. The same remarks apply to cotton plants bearing well-opened bolls where no such breakdown of the photosynthetic system is visible. In order to study this relationship it is necessary to make a periodic examination of the leaves of different nodes on a large number of plants. It should then be possible to establish the relation between the time of appearance of this internal symptom of the ' disease ' and the bad opening of the boll.

Experimental reproduction of the breakdown of chloroplasts

The two types of disintegration of the chloroplasts were reproduced experimentally in normal leaves in the following manner :—

If the daily increase in the size of the starch grains in the body of the chloroplasts, caused by some natural interference in the hydrolytic process,

results in the mechanical burst up of the chloroplasts, the same result could be obtained by interfering with the normal transport of carbohydrates from the leaves to the other parts of the plant by such means as ringing the stem. Cotton plants which did not show any such breakdown of the chloroplasts were selected for the experiment. Half the number of plants were ringed and the remainder were kept as controls. The leaves from the ringed and control plants were examined periodically. At the end of one month after ringing the leaves of the ringed plants showed a heavy accumulation of starch with distended cells (Plate XIV, fig. 5). The chloroplasts formed a network which was ruptured at many points. The leaves of the controls did not show an accumulation of starch or the breakdown of the chloroplasts.

The second type of disintegration of chloroplasts came to notice first in the leaves of plants in pots which had been treated with sodium chloride in an attempt to reproduce the symptoms of the 'disease'. In order to confirm this observation normal plants growing in a good field were treated with sodium chloride at the rate of 1500 lbs. per acre. The leaves of these plants were examined before the application of the salt and were found to be normal. A week after the application, the leaves showed the initial stages of disintegration of the chloroplasts (Plate XIV, fig. 6). Small cavities first appeared. The leaves became thick and fleshy and internally the mesophyll cells were elongated and showed turgidity. The chloroplasts later became vacuolated and sponge like with circular cavities. Tannins were produced in the cells (Plate XIV, fig. 7).

The increase in the thickness of the leaves and their fleshiness due to an increased water content were caused very probably by the absorption of the chlorine ions. Similar changes in the leaves of tobacco plant treated with sodium chloride were noticed by Garner, McMurtrey, Bowling and Moss [1930] and Wilson [1933]. The effect of sodium chloride on the cotton plant is investigated at present and the results will be communicated in a later publication.

Summary

The different stages of the disintegration of the chloroplasts in 4F Punjab-American cotton plant (*G. hirsutum*) are described. Disintegration is found to occur in two ways, produced by two different causes. The two types are classed here as (A) mechanical, (B) chemical. The mechanical burst-up of chloroplasts is caused by the daily increase in the size of the starch grains which remain unhydrolised, due probably to some interference in the enzymic activity of the cell. Chloroplasts bulge out and form a network with the starch

grains in the meshes. They also become ruptured, due to the pressure exerted by the starch grains. Substances giving reactions of tannins are formed later in the cells. The appearance of minute pores marks the starting point of the chemical type of disintegration of the chloroplasts. They first swell and large cavities appear in them. They then fuse together forming a spongy mass in the cells. Tannins develop and cover the dissolving chloroplasts. In some cases small granules appear before the small cavities are seen in the chloroplasts.

When the granules come out, cavities are left in the chloroplasts. Both types of disintegration are reproduced experimentally in normal leaves. The mechanical rupture of the chloroplasts is reproduced by interfering with the normal transport of carbohydrates from the leaves by ringing the stem. Chemical disintegration of the choloroplasts is brought about by the application of sodium chloride to the soil.

The breakdown of the photosynthetic apparatus is found to occur prematurely in the leaves of plants that develop the external symptoms of the 'disease' under investigation while no such disintegration of chloroplasts is noticed in the leaves of normal plants.

ACKNOWLEDGMENTS

Thanks of the author are due to Dr. T. G. Mason, F.R.S., for his suggestion to reproduce experimentally the mechanical rupture of the chloroplasts in normal leaves and to Mr. H. R. Stewart, Director of Agriculture, Punjab, for giving all facilities for work and for correcting the manuscript.

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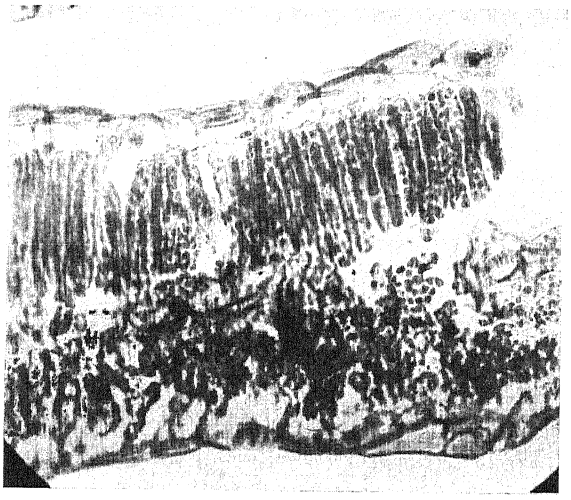


FIG. 1. Section of a leaf of 4F (*G. hirsutum*) showing normal chloroplasts (×150)

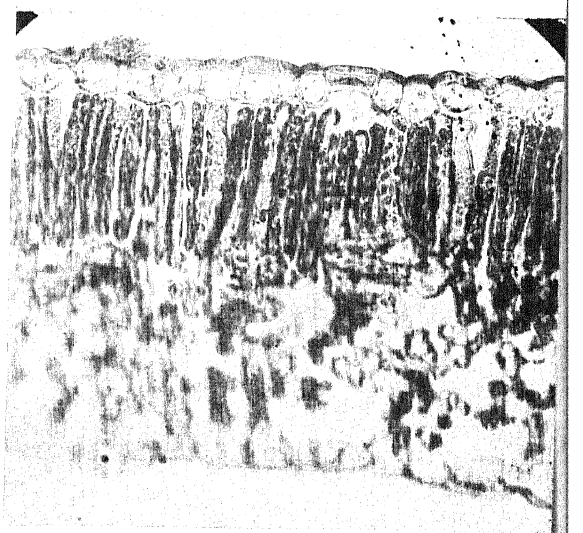


FIG. 2. Section of a leaf of 4F showing disintegrate chloroplasts with circular cavities (×150)

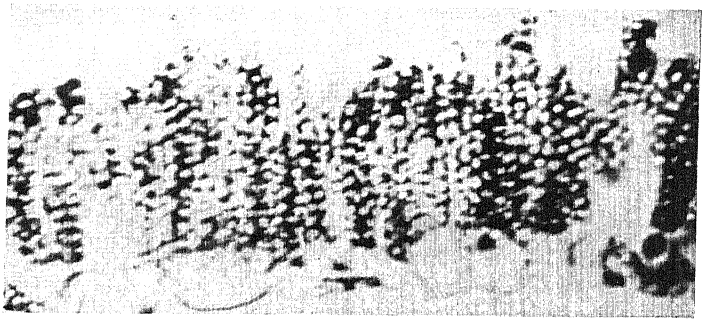


FIG. 3. Section of a leaf of 4F showing large starch grains (unstained) inside chloroplasts which are joined with one another and have formed a network (stained)

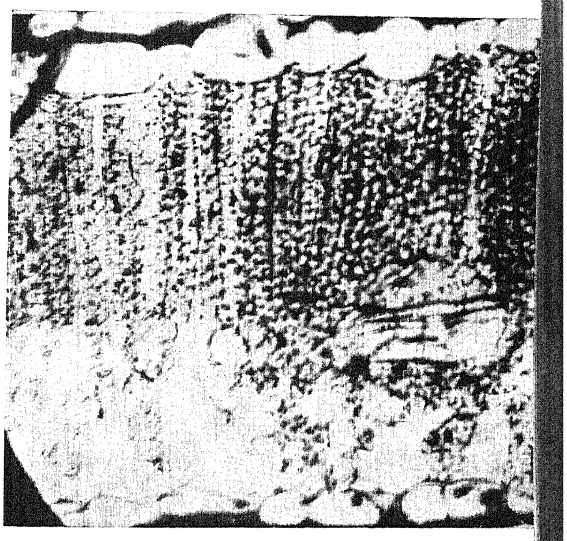


FIG. 5. Section of a leaf of 4F showing large starc grains inside chloroplasts (×400)

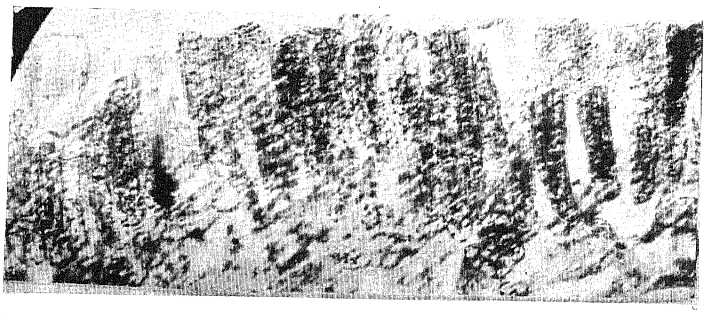
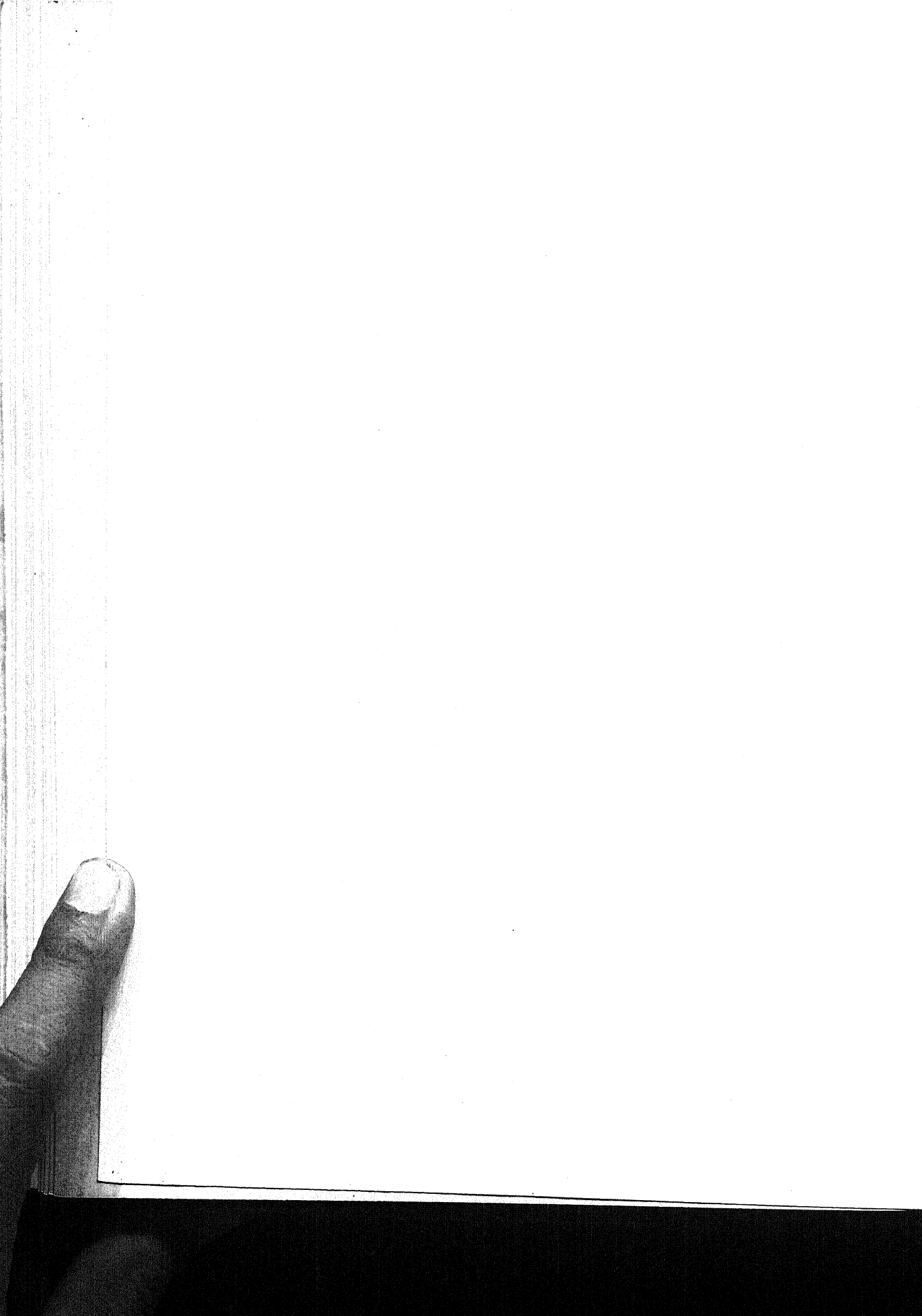


FIG. 4. The network of the chloroplasts after the removal of the starch grains by caustic potash (×350)



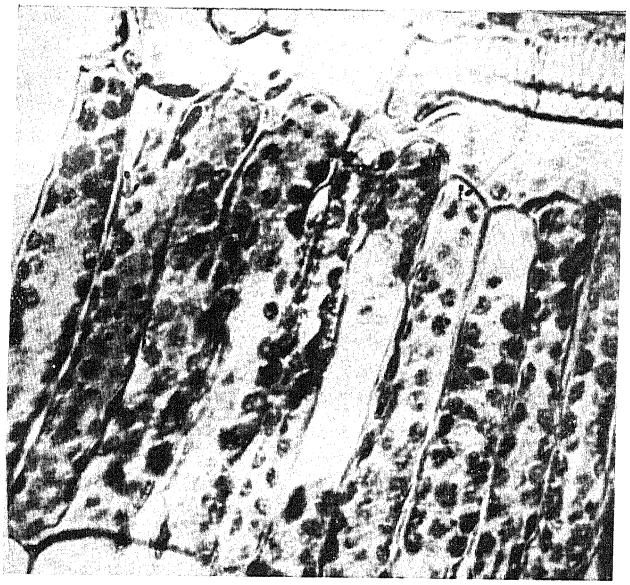


FIG. 1. Section of a leaf of 4F showing small granules in the body of the chloroplasts (×620)

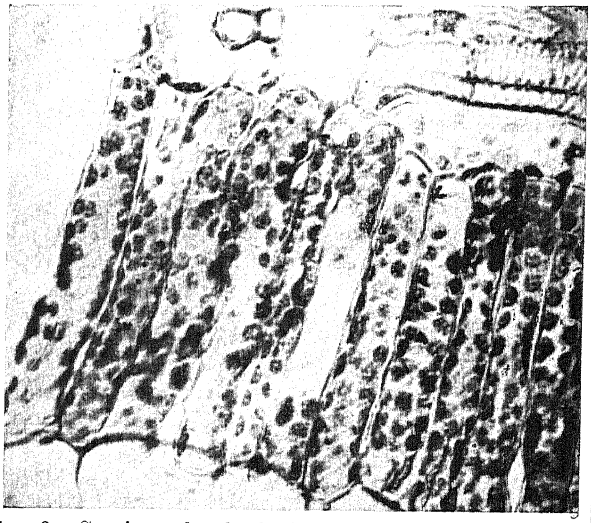


FIG. 2. Section of a leaf of 4F showing small granules in the body of the chloroplasts (×700)

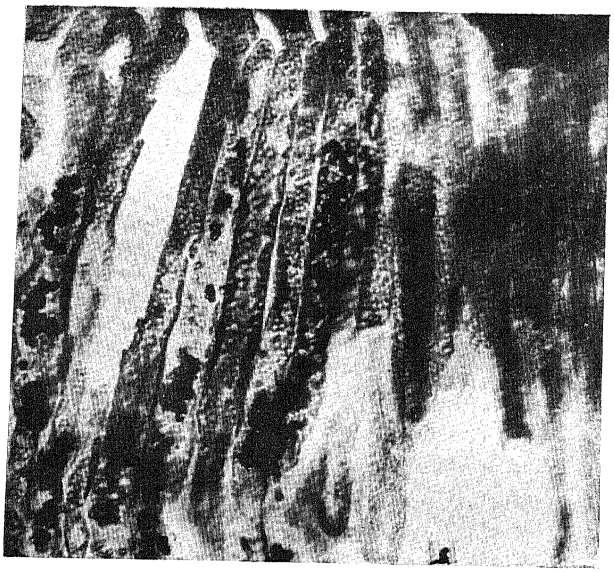


FIG. 3. Section of a leaf of an Egyptian cotton plant (Ashmoni), showing a swollen spongy mass of chloroplasts with large circular cavities (×500)

FIG. 4. Section of a leaf of an Egyptian cotton plant (Ashmoni), showing a swollen spongy mass of chloroplasts with large circular cavities (×600)

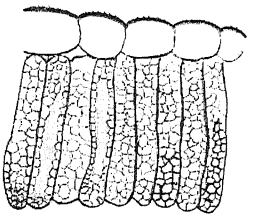


FIG. 1.

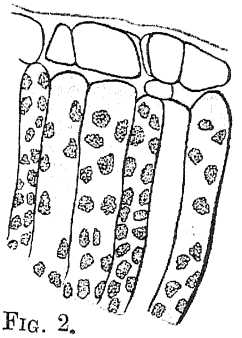


FIG. 2.

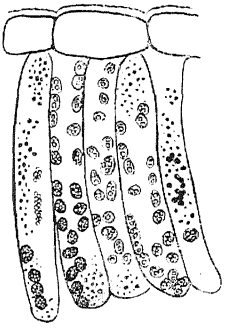


FIG. 6.

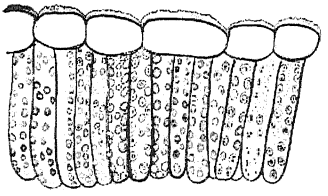


FIG. 4.

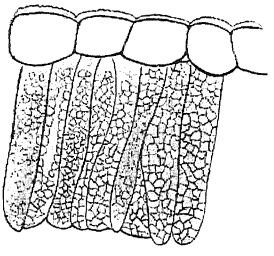


FIG. 5.

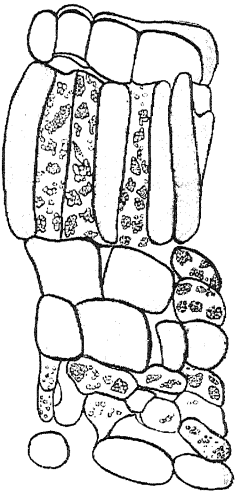


FIG. 3.

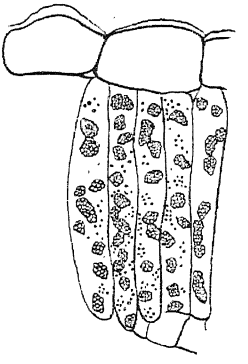


FIG. 7.

FIG. 1. Section of a leaf of 4F showing large starch grains surrounded by swollen chloroplasts, and tannin deposits (×200)

FIG. 2. Section of a leaf of a Cambodia cotton plant showing swollen sponge-like masses of chloroplasts with tannins (×250)

FIG. 3. Section of a leaf of 4F showing tannin deposits and the remains of disintegrated chloroplasts (×262)

FIG. 4. Section of a leaf of 4F showing small granules and cavities inside the chloroplasts (an early stage in disintegration) (×197)

FIG. 5. Section of a leaf of 4F showing accumulation of starch and tannins and the rupture of the chloroplasts produced by ringing the stem (×175)

FIG. 6. Section of a leaf of 4F treated with sodium chloride showing the early stage of chemical disintegration of chloroplasts (×232)

FIG. 7. Section of a leaf of 4F treated with sodium chloride showing swollen sponge-like masses of chloroplasts (×246)

II. FORMATION AND ACCUMULATION OF TANNINS IN LEAVES

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(With Plates XV-XVII)

INTRODUCTION

IN a preliminary note [Dastur, 1936], certain deposits in the cells of the leaves and roots were reported to be one of the abnormal features of Punjab-American cotton plants that produced prematurely cracked bolls with immature seeds and poor lint. A study was, therefore, made of the development of these deposits in the cells, to determine their chemical nature and their exact relation with the ' physiological disease '.

Much work has been done on the chemistry of the cotton plant (*G. hirsutum* aud *G. herbaceum*) and especially on the chemistry of the natural organic colouring matter of the flowers, leaves and the bark. Perkin [1899, 1909, 1916] extracted and isolated various organic compounds of the flavonoal group from the flowers. They were found to be glucosides of quercetin and gossypetrin. Quercimeritrin, isoquercitrin and quercitrin also were obtained from the leaves by Perkin [1909] and by Viehoever, Chernoff and Johns [1918]. According to Standford and Viehoever [1918] these glucosides are present in the internal resinous glands that are distributed in the petals, leaves and bark of the root and stem. These internal glands are found to be present in all parts of the 4F cotton plant grown in the Punjab. They contain a yellow colouring matter giving the microchemical reactions of quercitrin and related substances. The same glands also give the reactions of gossypol, C_{30} H_{30} O_8, which was first described by Perkin [1905] and later by various workers like Reaves and Valle [1932]. On account of the presence of gossypol, the contents of these glands turn a cherry red colour when treated with strong sulphuric acid. They are found in the largest number in the roots of 4F and are distributed in the cortex, medullary rays and phloem.

Power and Chestnut [1926] in their extensive studies on the volatile and non-volatile constituents of the cotton plant have also isolated a large number of organic substances from the alcoholic extract of the leaves and flowers. The main object of their investigation and those of other workers was to

isolate the substance which was supposed to attract the cotton bollworm insect that causes such great damage to the crop but these attempts did not meet with any success.

The above review of the chemical investigations of the cotton plant makes no mention of the presence of tannins in it. It is possible that the tannin deposits described below were either absent in the plant parts extracted by these investigators or that they escaped isolation from the large number of complex organic substances that were present in such extracts. The cells containing these deposits first came to notice when the leaves and roots of the 4F plant were being examined under microscope. In leaves and roots preserved in 4 per cent formalin they could be seen distinctly. The cells containing these deposits differed from those which constituted the internal glands described above. These deposits gave all the microchemical reactions of tannins in the fresh materials of leaves and roots. Without these colour reactions they could not be detected in fresh leaves or roots as these deposits are colourless in the natural condition. Similarly, they cannot be seen in plant material which has been preserved in alcohol as they are soluble in alcohol and so are extracted by it.

Investigation

The cells containing these deposits are found in all parts of the plant. The deposits are found in the cells of the mesophyll, the conducting parenchyma, cortex, medullary rays, xylem and phloem parenchymatous tissues, the pith and the pericarp of the unripe fruit.

The tannin deposits are present in the form of solution (Plate XV, figs. 1-4). They are also present in cells that contain starch grains. The starch grains are either partly or completely covered by them (Plate XV, fig. 3). When completely covered by tannins the starch-iodine reaction is not obtained. In some cells each individual starch grain is covered by a layer of tannin and all the reactions of tannin are given by the grains (Plate XV, fig. 3). The tannin deposits are also present in cells that contain minute granules of calcium oxalate (Plate XV, fig. 1).

In formalin-preserved material of roots and leaves the tannins are precipitated in an insoluble form. As such they cannot be dissolved by any organic solvent and are rendered insoluble even in cold acids or alkalies. The task of extracting the substance of these deposits became easy, once the above facts regarding their solubility and other properties came to be known after a great deal of preliminary work.

The detailed methods of extraction, purification and quantitative estimation have been worked out and will be published in a separate paper. The deposits in the cells are made up of tannins which are soluble in water. They form dark brown coloured insoluble phlobaphenes on treatment with acids. They are also precipitated by formaldehyde in presence of acids. They give all the reactions of tannins as described by Russel [1935] and appear to belong to the class of tannins known as phlobatannins.

In addition to the tannin deposits soluble in water and alcohol the cells in the bark of the root of the diseased plants contain cell deposits that are insoluble in these solvents. This is not the case with the roots of healthy plants. The presence of these two different types of deposits came to notice when the soluble tannin deposits were removed by alcohol. The presence of these cell deposits in the roots of diseased plants and their absence in those of healthy plants became clear in the material extracted with alcohol. These deposits consist mainly of precipitated protein matter and the uncrystallised salts of organic acids, like oxalic and tannic acids. In this investigation only the tannin deposits that are soluble in alcohol and water will be considered.

Having established the chemical nature of these cell deposits, investigations were conducted to determine their relation to the disease under investigation. A thorough and periodic examination of the tissues of normal and diseased 4F cotton plants showed that the tannins were present in varying degrees in the roots of all plants but the case was different with the leaves. In diseased plants the formation and accumulation of tannins begins to occur at an early stage in the growth of the plant while leaves from the normal plants do not show any accumulation of tannins at any stage. Between these two extremes—the totally diseased and normal conditions—plants may be found in which tannins accumulate in the leaves to a less extent during the fruiting stage. It was noticed also that a slight accumulation of tannins occurred at the fruiting stage in young leaves but the same plant did not show any such accumulations at a later stage.

In the diseased condition of the plant the accumulation of tannins in the leaves begins early in the vegetative phase and continues till the end of the season. The accumulation of starch, disintegration of chloroplasts and accumulation of tannins are all associated together in the diseased plants. These internal symptoms indicate the onset of the physiologically diseased condition which manifests itself in the early shedding of leaves and the bad opening of bolls. The relation between these internal symptoms and the ultimate bad opening of the bolls requires to be systematically investigated and established. Preliminary work in that direction showed that in order to establish these relationships it was necessary to examine the leaves from different nodes from a number of plants for these symptoms at regular intervals from an early stage of growth up to the fruiting time.

Of the three internal symptoms the tannin deposits are the easiest to detect under microscope, and therefore the daily examination of the large number of leaves that is necessary in order to investigate the relation between the internal symptoms of the disease and the final bad opening of the bolls is facilitated. Attention is, therefore, focussed on the accumulation of tannins in the leaves, and its relation to the external symptoms of the disease is here investigated.

From previous records of yields and general observations made on the opening of the bolls by the staff of the Lyallpur Agricultural Farm, three fields, where 4F cotton was to be sown in the season of 1937, were selected for the detailed observations referred to above. These fields are situated in

isolate the substance which was supposed to attract the cotton bollworm insect that causes such great damage to the crop but these attempts did not meet with any success.

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squares 29, 27 and 32 of the Farm, and are designated good, medium, and bad according to the condition of the crop during previous years. The soils in squares 29, 27 and 32 are considered good, medium and bad respectively for cotton cultivation. In the beginning a fourth field in square 26 was also selected but the plot, where the plants were labelled for observation, showed suppressed growth from an early stage, owing to other adverse soil conditions and, therefore, it is not included here. It was noticed that extreme bad opening occurs in plants that show normal growth until September. The *p*H and the mechanical composition of the soils up to a depth of six feet are given in Table I. No attempt will be made here, however, to discuss the bearing of the soil conditions on the observations made on the internal and external symptoms of the disease.

TABLE I

*p*H *and mechanical composition of the soils in the three fields*

	Square 29			Square 27			Square 32		
	Clay	Silt	Sand	Clay	Silt	Sand	Clay	Silt	Sand
1st foot . . .	14·3	28·1	55·3	16·0	18·4	65·2	11·5	13·0	76·0
2nd foot . . .	18·0	26·8	50·3	16·8	16·1	65·3	13·7	17·9	65·2
3rd foot . . .	20·5	24·7	48·5	19·3	12·9	65·9	14·3	20·2	62·2
4th foot . . .	17·1	29·2	44·8	16·7	14·9	72·4	15·4	24·1	53·4
5th foot . . .	19·2	26·8	45·1	15·0	15·8	64·5	13·3	20·5	58·1
6th foot . . .	14·8	20·2	58·1	14·0	15·5	65·8	16·5	18·9	62·4
*p*H range in the six feet of soil.	8·11-8·26			8·09-8·18			8·07-8·34		

Silt = Silt + fine silt fractions. Sand = Coarse + fine sand fractions.

After final thinning of the plants in these fields was done, ten plants in each field were labelled at random in two adjacent rows and the leaves from different nodes from each plant were periodically examined. The accumulation of tannins was first noticed in the leaves from plants in the bad soil of square 32, towards the end of July. From that date onwards a systematic examination, for tannins, of the leaves from nodes 4, 8, 10 and 12, counting from the apex, from the ten plants in each of the three fields was made at intervals of about three weeks. When microscopic examinations of the leaves for tannins were made, it soon became evident that both the amount and the extent of the accumulation of tannins varied in different leaves of the same plant on the same date. It further differed in the leaves of the same node from the apex of the ten plants in the same field as well as from the plants of different fields on the same date of sampling. The concentration of tannins in the cells and the number of mesophyll cells that contained these deposits varied from leaf to leaf. In order to get some idea of the comparative degree and extent of accumulations of tannins in the different leaves from the same and different plants from the three fields it was necessary to fix, and use some standards for comparison. After some preliminary trials the following procedure was adopted.

Standard slides of sections of leaves showing different degrees and extent of tannin accumulation were prepared. Firstly, sections of leaves showing zero accumulation of tannins and those completely filled with thick deposits of tannins were obtained and mounted. Then sections of leaves showing accumulations of tannins varying between these two limits were prepared.

Table II gives a description of each standard slide with the respective number of + signs employed to indicate the tannin status of the leaves. The use of + signs is here made to express differences in the degree and the extent of accumulation of tannins in the leaves.

In addition to the standard slides described above other standard slides indicating the degree and extent of tannin accumulation between any two successive standards were also prepared and kept handy for comparison. The differences in the accumulation of tannins indicated by these standard slides are not quantitative but must be regarded as merely comparative. Four +signs do not indicate that the tannin accumulation is four times the amount indicated by one + sign. The different signs only indicate the different stages of tannin accumulations in the leaves as seen under microscope.

TABLE II

Description of standard slides with + signs employed to indicate the tannin status of the leaves

Sign used	Description of standard slide
Nil	All cells devoid of tannin deposits (Plate XVI, fig. 1).
+	Tannin deposits in the spongy cells only (Plate XVI, fig. 2).
++	Thin tannin deposits in a few palisade and spongy cells. The palisade cells are not completely filled with tannins (Plate XVI, figs. 3, 4).
+++	Thick tannin deposits in some palisade cells and a large number of spongy cells (Plate XVII, fig. 1).
++++	Thick tannin deposits in the majority of palisade cells (65 per cent) and spongy cells (Plate XVII, figs. 2, 3).
+++++	Thick tannin deposits in almost all cells (Plate XVII, fig. 4).

Small pieces cut from similar positions on the leaves, of the 4th, 8th, 10th and 12th nodes, from each of the ten plants in the three fields were preserved separately in 4 per cent formalin every three weeks. Sections from the leaves of the fourth node from the ten plants in one field were cut separately. Sections were cut at three points from the piece of preserved leaf. A large number was examined and the values of tannin accumulations were noted by comparison with the standard slides. The average of these values was taken as the tannin status of the 4th leaf on one plant. Such ten values for the tannin accumulations were obtained from the leaf of the fourth node of the ten plants and the mean of the ten values was taken to indicate the condition of the 4th leaf for tannins in a field. The same procedure was adopted for leaves from the remaining nodes of the ten plants in the same field and for the different leaves of the ten plants in the other two fields. Such observations of the tannin status of the leaves of the ten plants in these fields were done five times during the cotton season. The results obtained on the 29th September, 1937, for the four different leaves of the ten plants in each field are given separately in Table III, in order to give an idea of the variations in the tannin accumulation in the four different leaves of the ten plants in each field.

TABLE III

Values for tannin accumulation for the leaves of ten plants in the three fields on 29th September 1937

Plant No.	Square 29 (good soil) Node from the apex of the leaves				Square 27 (medium soil) Node from the apex of the leaves				Square 32 (bad soil) Node from the apex of the leaves			
	4th	8th	10th	12th	4th	8th	10th	12th	4th	8th	10th	12th
P1	++	±	Traces	+	++	*Nil*	*Nil*	*Nil*	+++++	+++++	Shed	Shed
P2	*Nil*	Traces	*Nil*	*Nil*	+++	+	*Nil*	+	++++	+++	Shed	Shed
P3	+	Traces	*Nil*	*Nil*	++	+±	+±	Shed	++++	++++	+++++	Shed
P4	*Nil*	Traces	*Nil*	*Nil*	+	*Nil*	*Nil*	Shed	+++++	+++++	Shed	Shed
P5	+	Traces	*Nil*	*Nil*	++±	*Nil*	±	Shed	+++	Shed	Shed	Shed
P6	*Nil*	Traces	*Nil*	*Nil*	++±	+±	Traces	+	+++++	+++++	+++++	Shed
P7	Traces	Traces	Traces	Traces	++±	+	Traces	+	+++++	+++++	+++++	Shed
P8	*Nil*	Traces	+	*Nil*	+	++	+++	++	+++++	Shed	Shed	Shed
P9	±	Traces	*Nil*	Traces	++	+	Traces	Shed	++++	++++	++++	Shed
P10	*Nil*	*Nil*	Traces	Shed	++	++	++	++	+++++	Shed	Shed	Shed

The accumulation of tannins is least in all the leaves of the ten plants in the good field while it is very high in the leaves of plants in the bad field. Slight amounts of tannins are found to accumulate in the leaves of plants growing in the medium field. The shedding of the leaves is also greatest in the plants from the bad field. It started from the middle of August onwards, while such shedding did not occur in plants in the good field.

The average values of tannins for the ten leaves of each node in the plants in the three fields for the five different dates are stated in Table IV. They represent the tannin status of the four different leaves of the plants on each date of sampling. (The shed leaves were not examined for tannins. In that case the average value of the number of leaves that remained on the ten plants is taken). The differences in the tannin accumulation in leaves of the three fields are obvious. In the case of the good field the tannins accumulate in the spongy cells of some leaves, while in the medium soil the accumulation occurs in some palisade cells as well. All the leaves of the bad field show large accumulations of tannins. These examinations of the leaves of the different nodes and at different dates in the three fields suggest that the tannin-like substances are produced in all cases but that they are used up probably as respiratory material, in the leaves of plants from the good field while they remain accumulated in the leaves of plants growing in the bad field.

Records of the total number of bolls produced by each of the ten plants were kept. The percentages of badly opened and well-opened bolls were also determined. A boll is regarded as badly opened when the majority of the seeds in the bolls is immature and the lint is weak. The weight of seed cotton per boll and the boll weights, *i.e.*, the number of bolls required to make one seer of seed cotton, were also determined. These results are stated in Table V. The averages of the ten plants are also given. There is no real bad opening of the bolls in the plants in the good field. The figures of badly opened bolls represent the stained bolls due to insect damage as in P_2 (Table V). Very few bolls opened badly in the case of plants in the medium soil while the percentage of badly opened bolls in the bad field varied from 73 to 100. The number of bolls produced per plant was also in the same order in the three fields. The weight of seed cotton per boll in the bad field was one fourth of that in the good soil, thus indicating the immaturity of seeds in the former. Consequently, the boll weights are very high in the bad field. The observations made on shedding of leaves showed that shedding began as early as August in these plants. All leaves up to the 10th node from the apex of one plant (P_{10}) were shed on the 23rd August, 1938, and up to the 12th node in another three plants (P_1, P_5 and P_9).

TABLE IV

Average values for tannin accumulations in the leaves of plants in good, medium and poor fields (each value is an average of ten leaves or less)

Date (1937)	Node of the leaf from the apex	Square 29 (good soil)	Square 27 (medium soil)	Square 32 (poor soil)
1st August	4th leaf	±	+±	+++
	8th ,,	Traces	+	+++
	10th ,,	Traces	+±	++
	12th ,,	Traces	+±	+
23rd ,,	4th ,,	Traces	+±	++++
	8th ,,	+	++	+++±
	10th ,,	+	++	+++
	12th ,,	+	±	+++±
7th Sept.	4th ,,	*Nil*	*Nil*	++++
	8th ,,	*Nil*	+	++++
	10th ,,	Traces	++±	+++++
	12th ,,	Traces	+	+++++
29th ,,	4th ,,	±	++	++++±
	8th ,,	Traces	+	++++
	10th ,,	Traces	+	++++±
	12th ,,	Traces	+	Shed
25th October	4th ,,	+	++	+++++
	8th ,,	Traces	±	+++++
	10th ,,	+	+	Shed
	12th ,,	Shed	Shed	Shed

TABLE V

Percentage of badly opened bolls, boll weights and number of bolls per plant in the three fields

	Total number of bolls			Percentage of badly opened bolls			Weight of seed cotton in grams per boll			Boll weights (number of bolls required to make one seer of seed cotton)		
	Good	Medium	Poor	Good	Medium	Poor	Good	Medium	Poor	Good	Medium	Poor
P1 . . .	64	75	13	1·5	18	84	3·5	1·5	0·65	257	600	1384
P2 . . .	91	132	43	16*	7	90	1·6	2·6	0·63	562	346	1451
P3 . . .	62	34	57	3	11	93	2·5	2·0	0·68	360	450	1323
P4 . . .	148	58	169	3	15	80	2·4	2·1	0·90	375	428	1000
P5 . . .	111	41	10	1	5	100	2·9	2·2	0·25	310	409	3600
P6 . . .	91	77	56	1	5	92	2·5	2·3	0·57	360	391	1578
P7 . . .	94	77	39	4	5	92	2·8	2·3	0·52	321	391	1726
P8 . .	140	28	14	2	21*	100	2·3	1·9	0·42	391	473	2143
P9 . . .	151	69	26	10	1·4	73	1·9	2·0	0·50	473	450	1800
P10 .	89	98	2	3	7	100	2·7	2·0	0·50	333	450	1800
Average for 10 plants	104	68·9	43	4·9	8·8	86	2·48	2·18	0·70	365	413	1289

*Most of these bolls are badly opened due to injury caused by the insects.

The results of tannin accumulations in the leaves of these plants in the bad field clearly indicate that they suffer from some physiological disorder which gives rise to bad opening and consequently low yields. This disorder is manifested in all the leaves by the presence of thick tannin deposits from August onwards. No such accumulations of tannins are noticed in the leaves from plants in the good field. Thus thick tannin deposits in the cells of the leaves can now be regarded as a definite internal symptom of the ' physiological disease ' of bad opening.

The total yields of the three fields were also recorded. They were about 21, 17 and 4 maunds of seed cotton per acre for the good, medium and the bad field, respectively.

The actual yield of plants in a field depends upon a number of factors and when the plants suffer from this ' physiological disease ', the yield varies according to the growth and vigour of plants in that field. All the ten plants in the bad field show accumulation of tannins to an equal extent as far as microscopic observations go, but the number of bolls produced by each plant and the yields obtained vary to a very great extent. The number of bolls produced varied from 2 to 169 and yield of seed cotton from 1 gram to 152 grams. The same holds good for plant in the good field, the yields varying from 150 to 360 grams. Thus the presence of tannins in the leaves from an early stage indicates that the plant is not normal and suffers from some physiological disturbance. Consequently, the yield given by that plant may be expected to be less than it would be in the absence of this disease.

It is recently found that the tannin-like substances found in the leaves of 4F plants are chemically of two types. The differences between the two types become apparent when the fresh leaves are extracted with water and tested for tannins. The one type described above, which is found in the leaves of ' diseased ' plants, gives blue colouration with osmic acid and a reddish brown precipitate with formaldehyde and hydrochloric acid. The second type does not give this reaction with osmic acid and gives a yellowish precipitate with formaldehyde and hydrochloric acid. The relationships found between the accumulation of tannins in leaves and the bad opening of the bolls discussed above refer to the first type only.

The second type of substance which also gets precipitated with formaldehyde in the cells of the leaves is found in plants which are growing on heavy soils. The growth of plants in such cases is suppressed, as was the case in the leaves of plants in square 26 referred to above, where the cells showed the deposits in the leaves preserved in formalin. In the beginning they could not be differentiated from the tannins of the first type but later the differences in their chemical nature came to be noticed. The chemical nature of this substance and its relation to the ' disease ' under investigation is investigated.

It may be mentioned that in normal and healthy plants the accumulation of any of these two substances in the cells of the leaves is not found to occur

while any derangement in the metabolic activities of the plant, it appears, leads to the accumulation of any one of the two types of substances in the leaves.

Conclusions

In extreme cases of the ' physiological disease ' of bad opening of the bolls tannins begin to accumulate in all the leaves of the plant from an early stage while no such accumulations occur in the leaves of healthy plants. Tannin accumulation in the leaves from an early stage of growth is, therefore, an internal index of the disease. The diseased plants do not show externally any symptoms of the disease during the vegetative phase and hence an early diagnosis of the onset of the disease is possible only by microscopic examination of the leaves of such plants.

The microscopic method of determining the diseased condition is both tedious and lengthy and it is not possible to make use of this method on a large scale. From a practical point of view it would be advantageous to use this plant index in finding out at an early stage the condition of the crop in a field. It is, therefore, necessary to replace the present microscopic method, which further is more or less qualitative and not quantitative, by a more rapid and easy one. It cannot be applied to determine the different degrees of intensity of the disease. A suitable method had to be devised by which different intensities of the diseased condition could be determined. A colorimetric method was, therefore, worked out and is being applied at present to test the relationship between this internal symptom and the bad opening of the bolls.

Summary

The chemical nature of the cell deposits in the leaves and roots of 4F Punjab-American cotton plants mentioned by the author in this Journal (Vol. 6, pp. 377-9, 1936) is determined. These deposits are made up of substances classed as tannins or their derivatives.

The accumulation of tannins giving the osmic acid test is found to occur in the leaves of plants which later develop the external symptoms of the disease, *viz.*, the reddening and shedding of the leaves and the bad opening of bolls. Such accumulation of tannins is not found to occur at any stage in leaves of normal plants.

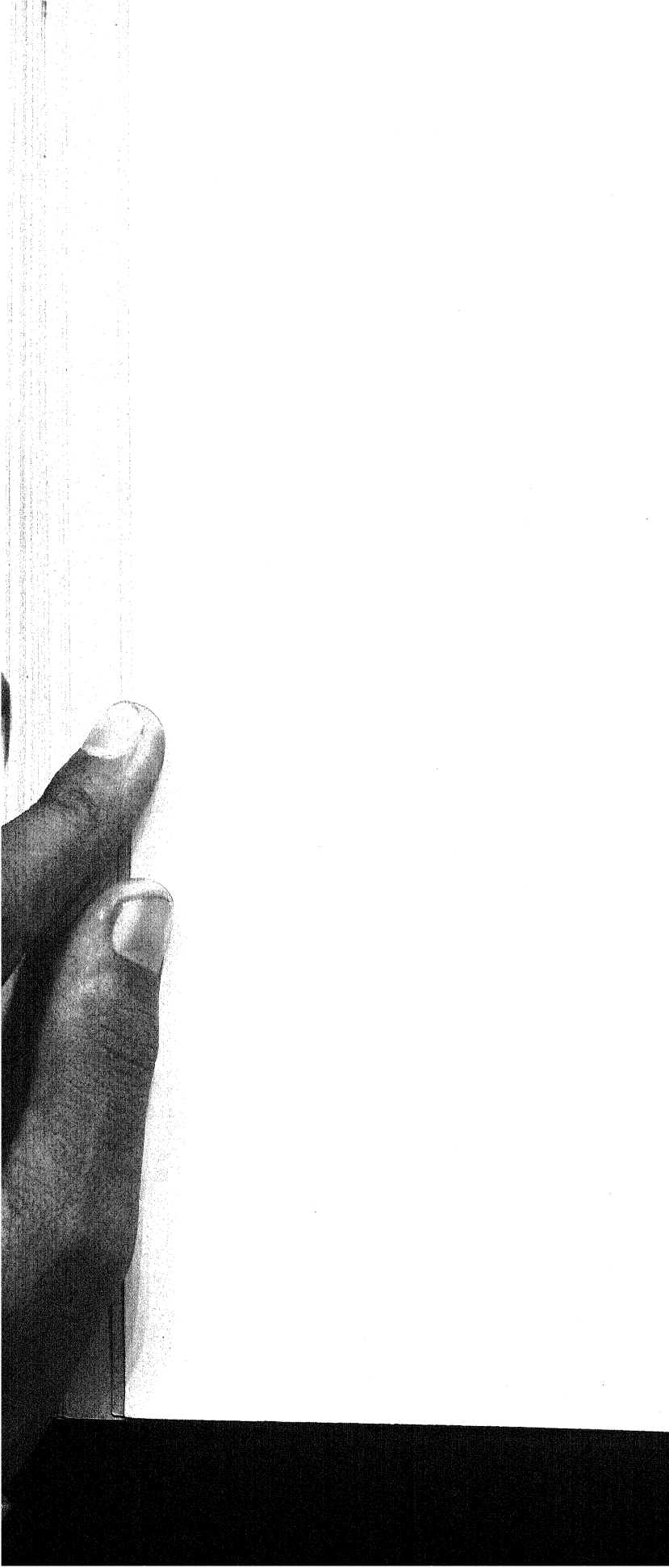
The relationship between the accumulation of tannins in leaves and the final bad opening of bolls on the diseased plants is established by a systematic and detailed examination of different leaves of ten plants in three fields where the soils are classed as good, medium and bad, according to the condition of the cotton crop in previous years. The leaves of plants from the bad soil showed the largest accumulation of tannins from an early stage of growth. On an average the percentage of badly opened bolls on the plants was 85. The leaves from plants grown on the good soil showed no accumulation of

FIG. 1. Section of a root showing the cells of the bark containing tannin deposits

FIG. 2. Cells from the bark of a root of 4F showing tannin deposits (×260)

FIG. 4. Cells from the midrib of a leaf showing tannin deposits. Enlarged parenchymatous cells show the swollen spindle-shaped nuclei

FIG. 3. Cells from the bark of a root of a 4F cotton plant showing starch grains covered with tannins (×400)



PHOTOMICROGRAPHS OF STANDARD SLIDES FOR TANNIN ACCUMULATIONS

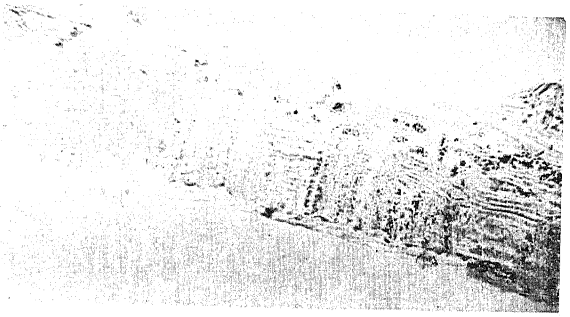


FIG. 1. Section of an old leaf of 4F cotton plant free from tannin (*nil*)

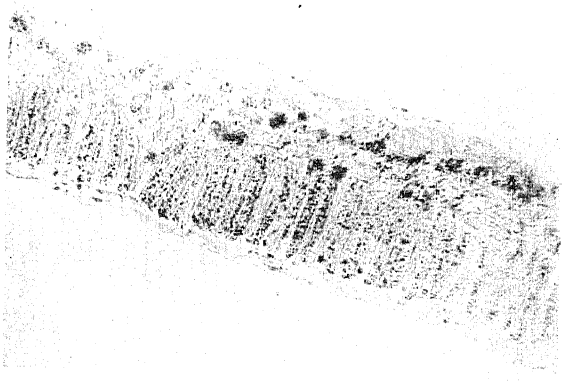


FIG. 2. Section of a leaf of a 4F cotton plant showing tannin deposits in the spongy cells (+)

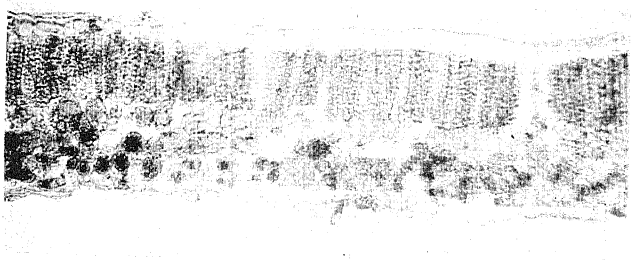


FIG. 3. Section of a leaf of a 4F plant, showing thin tannin deposits in a few palisade and spongy cells. The palisade cells are not completely filled with tannins (++)

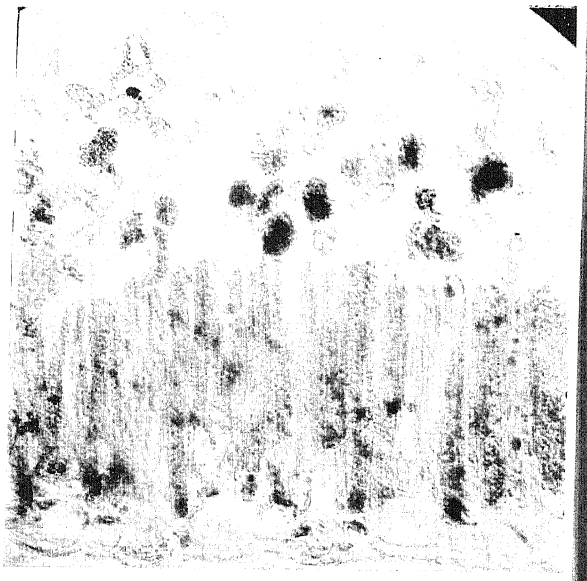
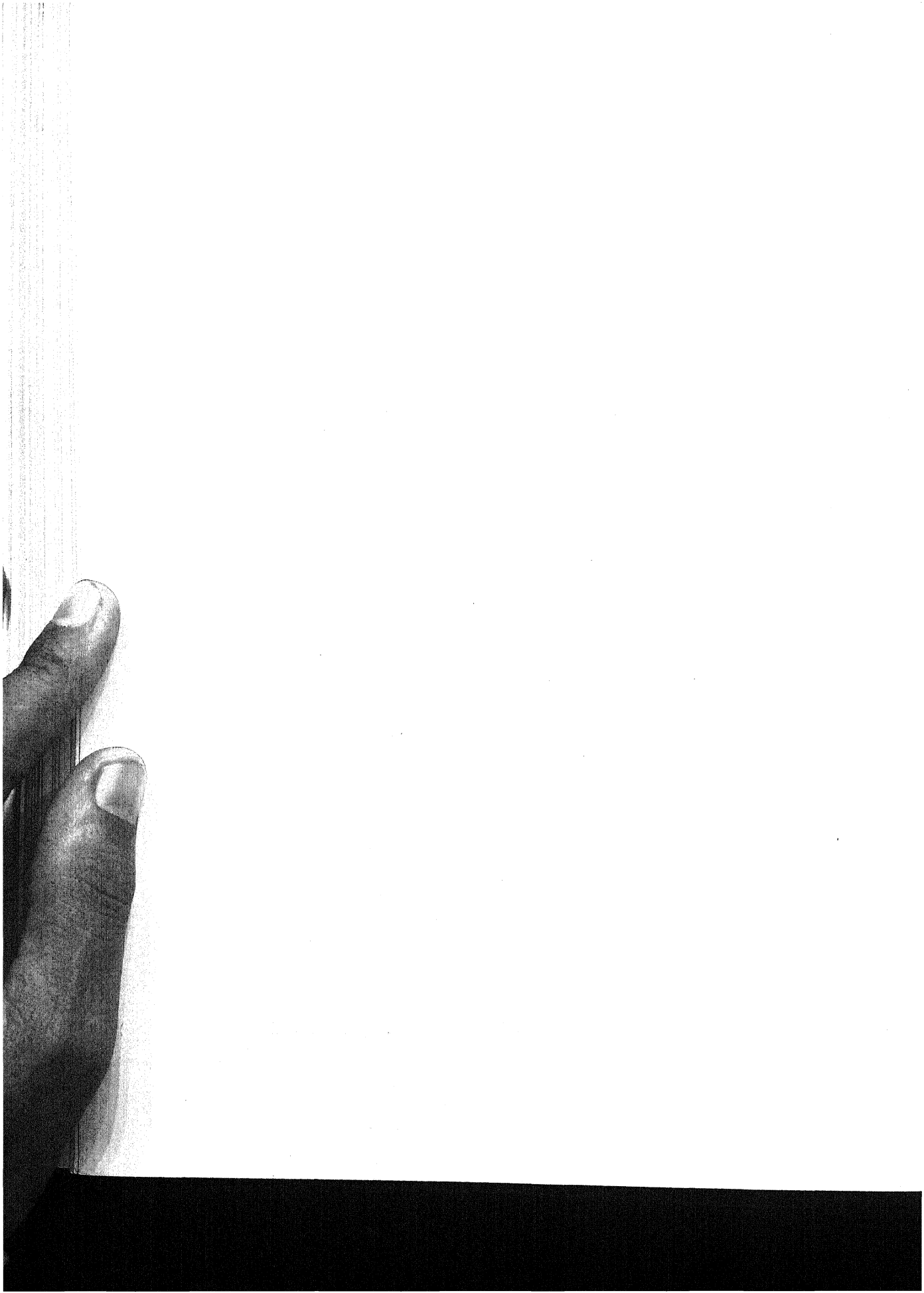


FIG. 4. FIG. 3 more highly magnified (++)



PHOTOMICROGRAPHS OF STANDARD SLIDES SHOWING TANNIN ACCUMULATION

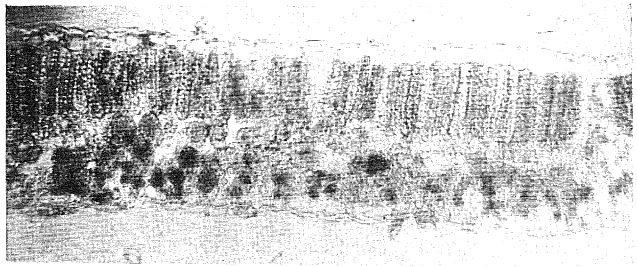


FIG. 1. Section of a leaf of a 4F cotton plant, showing thick tannin deposits in some palisade and in a larger number of spongy cells (+++)

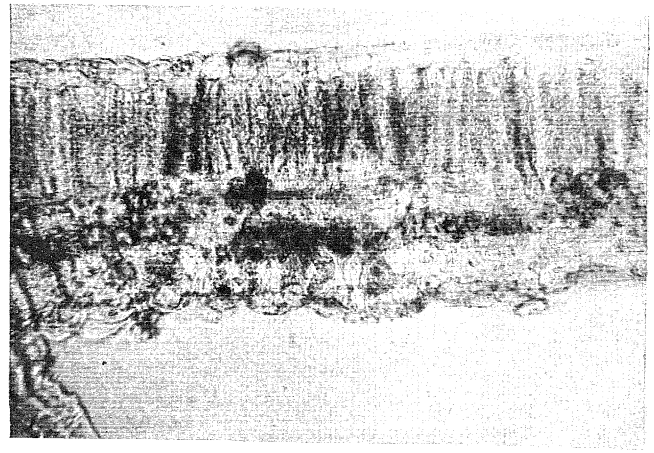


FIG. 2. Section of a leaf of a 4F plant showing thick tannin deposits in a majority (65 per cent) of palisade cells and spongy cells (++++)

FIG. 3. FIG. 2 more magnified (++++)

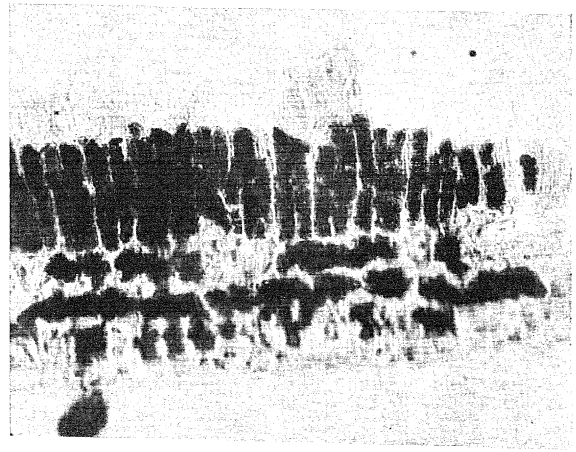


FIG. 4. Section of a leaf of a 4F cotton plant showing thick tannin deposits in almost all cells (+++++)

tannins at any stage of growth and no bad opening of bolls occurred on these plants. The leaves from plants from the medium soil showed slight accumulation with about 10 per cent of bolls badly opened.

The yields of plants from the three fields were in the same order. It is important to note that the yield given by a plant showing this internal symptom of the disease depends also on other soil conditions.

The accumulation of tannins in leaves from an early stage can now be safely regarded as an index of an abnormal and unhealthy condition of the plant.

The differences in the tannin accumulation of the leaves given in this paper are roughly comparative. An attempt is now being made to determine these differences by a quantitative method and to find out whether any quantitative relation exists between the accumulation of tannins in leaves and the different degrees of bad opening noticed in different plants.

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VARIATIONS IN THE MEASURABLE CHARACTERS OF COTTON FIBRE

I. VARIATION WITH RESPECT TO THE LENGTH OF THE FIBRE

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(With eight text-figures)

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I. Introduction

It is a well-known fact that the individual fibres that go to make up a sample of cotton vary considerably in their physical properties. These variations are brought about by numerous causes both genetic and environmental. They have been dealt with in detail by Turner [1929], and others [Editorial, 1930]. In the present work and in the ones that are to follow, it is the object of the writer to study the extent of variation that is caused by each of the

different factors enumerated by Turner. The variation of the fibre-weight with respect to length was dealt with in a previous paper [Iyengar and Turner, 1930] and the present work deals with the variation of the other properties with respect to length.

II. Material and sampling

The material used in the present enquiry consisted of four samples of cotton, one belonging to strain Karunganni C 7 (*G. indicum*), another to strain 2405 (*G. herbaceum*) and the remaining two to strain Co 2 (*G. hirsutum*), the difference between the last two being, that one was collected from the irrigated and the other from the rain-fed plots of a randomised replicated experiment laid out for a study of the effects of irrigation.

The sampling was made with the object of having it as uniform as possible by eliminating the extraneous influences. The variation between samples of different plants was minimised by confining the sampling to as few plants as were barely necessary, the variation due to the age of the plant was minimised by taking the picking on a particular day, and that due to the composition of bolls and locks by taking only those bolls that were three locked and which contained the same number of seeds in each lock. All locks with one or more aborted seeds were discarded. The fourth seed of each lock alone was selected to form the sample.

III. Experimental methods

The seeds were carefully delinted by hand so as not to break any of the fibres and out of the lint obtained, a sliver was prepared by using the drawbox of the Balls Sorter equipment. After arranging the fibres from the sliver on a Baer Sorter, the fibres of different length-grades were extracted in the manner described in the previous paper [Iyengar and Turner, 1930]. A tuft of fibres containing approximately the required number of fibres of a particular length-grade was placed on a piece of plush and the fibres removed from them were used for the determination of the following properties :—

1. Number of convolutions per unit length of the fibre,
2. Ribbon-width—maximum, minimum and mean,
3. Swollen fibre-diameter,
4. Swollen wall-thickness, and
5. Immaturity.

Six consecutive length-classes differing by 1/8 in. namely, from 10/8 in. to 5/8 in., were examined in either of the samples of Co 2 and five (9/8 to 5/8 in.) in each of strains Karunganni C 7 and 2405.

Regarding the number of fibres to be tested for the determination of the different properties, Koshal and Turner [1930] have stated that it should be 300 for ribbon-width and 500 for convolutions. As the sample from which these numbers were derived was a bulk material, it was considered that in the present set of samples, where causes of variation were minimised and where the material under test consisted of a small tuft of fibres of uniform length, the variation would not be so high. Further the work being arduous, 200 fibres only were examined for each property. For the estimation of maturity, however, 200 more fibres were tested except for strain 2405*. The results obtained, however, indicate that the variability does not always decrease as anticipated, though in some cases the standard errors were small enough. The number of fibres tested should, therefore, be considered not quite adequate in all cases.

The fibres were mounted with seccotine in the manner usually followed for the determination of the convolutions and ribbon-width. Generally ten fibres were mounted on each slide taking care to have at least one of their ends nearly in a straight line, so that they may be covered with a coverslip later.

By moving the slide under the microscope (magnification $\times 260$) the length of the fibres and the total number of convolutions along the whole length were determined. Neither the direction of the convolutions nor the number of reversals was taken into account. The maximum ribbon-width and the minimum ribbon-width [Barritt, 1930] in the immediate neighbourhood of the maximum, were then determined at ten different places, spaced nearly equally apart along the length of the whole fibre. When all the fibres on the slide had been examined, they were fixed at the two ends by means of the wax used for fixing the fibres for strength determination and were covered with a suitable coverslip. The fibres were then irrigated with 18 per cent solution of caustic soda and examined half an hour † later under a higher magnification ($\times 590$).

It is well known that under this treatment a ripe fibre gets fully swollen and assumes a rod-like appearance. But an immature fibre shows a tubular structure with an internal canal. The thickness of the wall varies according to the degree of maturity of the fibre. The diameter of the fibre (D) and the diameter of the internal canal (d) were measured in the microscope at ten equal intervals along the length of the whole fibre, as was done in the case of

* As there was negligible immaturity in this strain, 200 fibres were deemed sufficient.

† Any amount of this resting period, above a minimum, was found to have no effect on the dimensions measured, under the magnification used, provided the solution did not evaporate to dryness.

the ribbon-width. D, then, represents the diameter of the fibre-cell and $D-d$, twice the wall-thickness of the swollen fibre.

The ratio of the fibre-diameter to wall-thickness of the swollen hair was used to estimate the maturity of the fibre. In order to determine the exact limits of the ratios between which fibres of the different maturity classes may be said to lie, the fibre was carefully scrutinised both in the raw and the swollen state and its condition of maturity, as to whether it can be classed as ripe, dead or thin-walled, was recorded.

It may be mentioned here that though most of the fibres generally exhibited uniformity in the degree of ripeness along their length, there were some fibres which showed wide variations *. Taking only those fibres that exhibited uniformity, a study of the distribution of the ratio of fibre-diameter to wall-thickness was made in the three maturity classes, in all the four cottons. The results obtained in the case of the rain-fed sample of Co 2 is recorded in Table I and Fig. 1 to serve as an example. The distributions in the other cottons also indicated similar limits of the ratio for the three maturity classes. Even in the strain 2405, where the immature fibres are very few, the same limits are found to be adhered to.

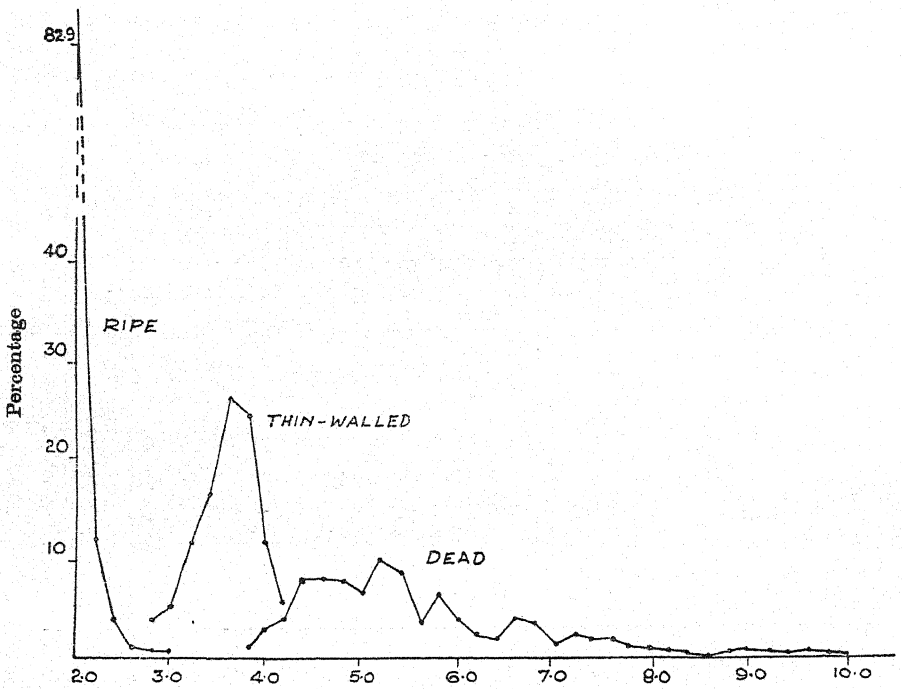

FIG. 1.

* Such a feature has also been noticed by Gulati and Ahmad [1935].

TABLE I

Ratio of fibre-diameter to wall-thickness

Ripe fibres			Thin-walled fibres			Dead-fibres		
Class interval	No.	Per-centage	Class interval	No.	Per-centage	Class interval	No.	Per-centage
2·00—2·10	417	82·9	2·71—2·90	2	3·2	3·71—3·90	2	0·6
2·11—2·30	60	11·9	2·91—3·10	3	4·8	3·91—4·10	9	2·8
2·31—2·50	17	3·4	3·11—3·30	7	11·3	4·11—4·30	17	5·4
2·51—2·70	5	1·0	3·31—3·50	10	16·2	4·31—4·50	23	7·3
2·71—2·90	2	0·4	3·51—3·70	16	25·8	4·51—4·70	24	7·6
2·91—3·10	2	0·4	3·71—3·90	15	24·2	4·71—4·90	23	7·3
			3·91—4·10	7	11·3	4·91—5·10	20	6·3
			4·11—4·30	2	3·2	5·11—5·30	31	9·8
						5·31—5·50	27	8·5
						5·51—5·70	10	3·2
						5·71—5·90	20	6·3
						5·91—6·10	12	3·8
						6·11—6·30	7	2·2
						6·31—6·50	6	1·9
						6·51—6·70	12	3·8
						6·71—6·90	10	3·2
						6·91—7·10	5	1·6
						7·11—7·30	6	1·9
						7·31—7·50	7	2·2
						7·51—7·70	6	1·9
						7·71—7·90	4	1·3
						7·91—8·10	3	0·9

TABLE I—*contd.*

Ripe fibres			Thin-walled fibres			Dead fibres		
Class interval	No.	Per-centage	Class interval	No.	Per-centage	Class interval	No.	Per-centage
						8·11—8·30	2	0·6
						8·31—8·50	1	0·3
						8·51—8·70	0	0·0
						8·71—8·90	1	0·3
						8·91—9·10	3	0·9
						9·11—9·30	2	0·6
						9·31—9·50	2	0·6
						9·51—9·70	1	0·3
						9·71—9·90	2	0·6
						9·91—10·10	1	0·3
						Greater than 10·10	17	5·4
Total .	503			62			316	

It will be seen that the ripe fibres range from 2·0 to 3·0; but most of the values lie in the neighbourhood of 2·0 and there is no value beyond 3·0. The dead fibres start at 3·8 and the value of the ratio rises to as high as 12. Only two fibres, or 0·6 per cent of the total number in this class, fall below 4·0. The thin-walled fibres lie between 3·0 and 4·0 with a little tail on either side and with mode at 3·6. Thus the ranges between which fibres of the three types of maturity lie seem to be circumscribed. The ripe fibres may be said to range from 2·0 to 3·0, the thin-walled fibres from 3·0 to 4·0 and the dead beyond 4·0. Employing these limits as standards, the percentages of the fibres in the different classes have been calculated.

Peirce and Lord [1934] have also considered this aspect and they have stated that "the limits of the thin-walled class are defined roughly by the

values 0·2—0·35 for the ratio of wall-thickness to ribbon-width" *which when converted into the ratio adopted in the present enquiry † work out to be 2·86 to 5·0. The lower limit is practically the same as the one found here, namely, 3·0 but the upper one is a little higher. But from a comparison of the ratios with the actual degree of maturity of the fibres, it was found, as shown in the foregoing distributions, that 4·0 was the more reliable limit in the samples handled here.

It may also be mentioned that these workers have used the cut section for their measurements. They have stated that they used "a method designed to secure a minimum of distortion or obliquity" but the error due to this cannot be as completely eliminated as in the present method, where it is non-existent. Moreover, the sections show the value at a particular portion of the fibre which may not be the representative behaviour along the entire length, as it is in the present case. These differences between the methods may possibly account for the higher value obtained by them for the upper limit of the thin-walled fibres, presuming that there has been no personal factor influencing the fixing of the border itself.

The significance of the differences between the maturity percentages has been tested by the use of the standard error, S, calculated according to the formula $S^2=\frac{pq}{n}$, where p and q represent the proportions of the fibres falling within and outside a class respectively, and n, the number of fibres examined [Yule, 1929].

IV. Discussion of results

Before taking up the discussion of the results proper, it may be stated that the normality or otherwise of any distribution, stated in the following discussion, is as judged visually from the frequency polygons only; no statistical tests for judging normality or otherwise have been made. The standard errors and the values of significance have been calculated wherever the distribution can be deemed nearly normal.

The variation of all the properties examined among the different length-grades may be seen at a glance in Table II.

(*a*) *Immaturity*

Before taking up the variations in the physical properties it would be appropriate to discuss here the variations in the maturity of fibres as this character is observed to influence almost all the other properties.

* From the statement "after separating the three groups of the immaturity test" it is assumed that these authors have used fibres swollen in caustic soda.

† As the present work was started in 1933, much earlier than the publication of the work of Peirce and Lord and as the ratio of fibre-diameter to wall-thickness was chosen from the commencement, it has been employed instead of the inverse ratio of wall-thickness to diameter used by these authors.

TABLE

Summary

Cotton	Length				Convolutions per cm.			Ribbon-width					
	Expected in		Actual mean in mm.	S. E.	All fibres	Ripe fibres only	S. E. of (7)	Maximum	S. E.	Minimum	S. E.	Mean	S. E.
	inch	mm.											
1	2	3	4	5	6	7	8	9	10	11	12	13	14
Co 2 irrigated	10/8	31·8	30·6	0·148	26·4	43·5	1·38	20·5	0·185	8·6		14·7	0·092
	9/8	28·6	28·1	0·106	31·0	57·0	1·92	21·4	0·176	8·2		14·9	0·092
	8/8	25·4	25·6	0·131	43·2	63·7	1·61	21·3	0·199	9·1		14·9	0·106
	7/8	22·2	21·5	0·150	50·6	63·9	1·76	20·6	0·194	9·5		15·2	0·111
	6/8	19·1	17·6	0·163	47·0	66·8	1·98	21·4	0·240	9·0		15·3	0·143
	5/8	15·9	13·8	0·179	40·6	65·0	2·02	20 9	0·222	8·4		14·8	0·139
Co 2 Rain-fed	10/8	31·8	30·9	0·147	44·2	71·4	1·63	19·9	0·203	7·8		13·9	0·092
	9/8	28·6	28·6	0·118	44·3	74·5	2·37	20·8	0·194	7·5		14·2	0·092
	8/8	25·4	26·7	0·118	45·8	79·7	2·32	20·6	0·222	7·5		14·1	0·088
	7/8	22·2	22·6	0·144	59·8	79·6	2·15	20·8	0·203	8·7		14·9	0·106
	6/8	19·1	18·3	0·161	44·2	67·1	2·07	21·3	0·240	8·7		15·0	0·129
	5/8	15·9	14·9	0·185	48·4	70·2	2·76	21·7	0·264	7·6		15·0	0·143
Karunganni C 7.	9/8	28·6	28·5	0·115	38·8	46·8	1·32	17·2	0·139	8·3	0·111	13·3	0·078
	8/8	25·4	25·6	0·095	33·7	40·6	1·15	18·0	0·157	8·5	0·148	13·8	0·083
	7/8	22·2	22·8	0·106	31·6	38·9	1·10	19·1	0·217	8·6		14·3	0·088
	6/8	19·1	19·5	0·123	26·6	36·0	0·99	19·7	0·269	8·6		14·8	0·097
	5/8	15·9	15·4	0·156	20·0	31·2	1·06	21·1	0·287	8·3		15·3	0·092
Strain 2405	9/8	28·6	26·7	0·138	25·8	26·0	0·49	17·2	0·148	10·4	0·083	13·9	0·106
	8/8	25·4	24·7	0·086	31·7	32·3	0·58	17·6	0·129	10·7	0·079	14·2	0·097
	7/8	22·2	22·5	0·090	30·8	31·6	0·62	18·2	0·134	11·0	0·083	14·7	0·092
	6/8	19·1	19·5	0·095	28·4	29·2	0·63	17·8	0·148	11·2	6 111	14·6	0·097
	5/8	15·9	15·5	0·102	30·8	31·0	0·60	18·9	0·157	12·0	0.116	15.6	0·125

* Standard error based on 200 readings

II

of results

in μ						Immaturity (per cent)					
Max. / Min.	Fibre diameter	S. E.	Wall-thickness	S. E.	Col. 16 / Col. 18	Ripe	S. E.	Dead	S. E.	Thin-walled	S. E.
15	16	17	18	19	20	21	22	23	24	25	26
2·59	22·8	0·106	7·5		3·64	46	2·5	41	2·4	13	1·7
2·61	23·2	0·123	7·3		3·78	41	2·4	41	2·4	18	1·9
2·69	23·8	0·138	8·6		3·48	55	2·5	29	2·2	16	1·8
2·37	24·0	0·132	9·4		3·16	65	2·4	22	2·0	13	1·7
2·62	24·4	0·174	9·2		3·30	62	2·4	26	2·2	12	1·6
2·90	24·0	0·198	8·8		3·44	58	2·4	30	2·0	12	1·6
2·82	20·6	0·110	7·3		3·52	52	2·5	34	2·4	14	1·8
3·20	22·2	0·110	7·0		3·84	48	2·5	44	2·4	8	1·4
3·08	23·0	0·123	8·1		3·36	60	2·4	29	2·2	11	1·6
2·84	24·0	0·136	9·4		3·18	68	2·4	20	2·0	12	1·6
3·16	24·4	0·176	9·0		3·42	62	2·4	27	2·2	11	1·6
3·97	24·1	0·196	8·0		4·02	50	2·5	38	2·4	12	1·6
2·13	21·8	0·100	8·5		2·76	75	2·2	8	1·4	17	1·9
2·16	23·0	0·106	8·8		3·04	72	2·2	15	1·8	13	1·7
2·59	23·8	0·117	8·5		3·60	66	2·4	28	2·2	6	1·2
3·16	24·5	0·132	8·4		4·14	56	2·4	38	2·4	6	1·2
3·72	24·8	0·132	7·9		4·42	52	2·5	44	2·4	4	1·0
1·640	21·8	0·172*	10·7	0·089	2·058	99·5	0·5	0·0	0·0	0·5	0·5*
1·639	22·6	0·162	11·1	0·096	2·050	99·0	0·7	0·5	0·5	0·5	0·5
1·623	23·5	0·172	11·5	0·104	2·060	98·5	0·8	0·5	0·5	1·0	0·7
1·644	24·1	0·183	11·8	0·121	2·044	98·5	0·8	1·5	0·8	0·0	0·0
1·574	25·8	0·218	12·8	0·123	2·032	99·0	0·7	0·5	0·5	0·5	0·5

only for this cotton ; for the other three cottons on 400 readings.

It will be seen that in both the irrigated and rain-fed samples of Co 2, the ripe fibres decrease slightly from 10/8 in. to 9/8 in. after which they increase up to 7/8 in. and then again decrease (Table II, Col. 21 ; Fig. 2). The dead fibres, as should be expected, record an opposite tendency, there being a fall from 9/8 to 7/8 followed by a rise. Though the fall of maturity from the 10/8 in. group-length to the 9/8 in. is not significant, its persistance in both the samples of Co 2 and in the other tests described below should indicate to its real existence. In Karunganni the variation is in a single direction, there being a fall in the ripe and thin-walled fibres and a rise in the dead fibres with the decrease in length. Almost all the differences in the case of the dead fibres and many in the case of the ripe fibres are statistically significant. In the strain 2405 there is negligible immaturity with little variation. It will thus be seen that there are distinct variations of maturity with respect to length in some strains, the trend of variation depending upon the strain of cotton.

To have a comparative idea of the results obtained according to Clegg's method with those derived according to the present method, the fibres in the different length-grades were also examined by Clegg's method by testing five bunches of about hundred fibres each for each length. The results obtained are given in Table III.

On comparison with Table II, columns 21, 23 and 25 it will be seen that in most of the cases the differences between the values obtained according to the two methods are well within sampling errors. The trends of variation with respect to length, that can be deduced from these results are practically identical with those according to the other method (Figs. 2 and 3). It is remarkable to note that the very simple method adopted by Clegg gives results which are nearly as reliable as those got by the method which is very much more elaborate and time-absorbing.

With regard to the variation of the maturity in the different lengths, Underwood [1930] states that " As a general rule the short hairs contain higher proportions of immature cotton than the longer hairs." This can be conceived in a bulk sample badly ginned by machinery where many of the long immature fibres may break and thus increase the amount of immature cotton in the shorter lengths. In the present case the increase of the immature fibres in the shorter lengths in the strain Co 2, especially after a decrease up to the 7/8 in. length, may lead one to suspect that it may be due to a similar cause, in view of the fact that the proportion of the immature fibres in the longest length is very great in this cotton. But since great care was taken against any breakage during the preparatory processes the influence of this factor should not be considerable.

TABLE III

Immaturity percentage according to Clegg's method

	Group length in 1/8 in.	Co 2 irrigated		Co 2 rain-fed		Karunganni	
		Value	S. E.	Value	S. E.	Value	S. E.
Ripe	10	50	2·2	51	2·2	..	..
	9	49	2·2	45	2·2	82	1·7
	8	61	2·2	62	2·2	74	2·0
	7	62	2·2	62	2·2	59	2·2
	6	58	2·2	56	2·2	50	2·2
	5	47	2·2	52	2·2	46	2·2
Dead	10	32	2·1	31	2·1	..	..
	9	33	2·1	38	2·2	9	1·3
	8	24	1·9	25	1·9	17	1·7
	7	27	2·0	24	1·9	30	2·1
	6	30	2·1	27	2·0	37	2·1
	5	42	2·2	33	2·1	41	2·2
Thin-walled	10	18	1·7	18	1·7	..	..
	9	18	1·7	17	1·7	9	1·3
	8	15	1·7	13	1·5	9	1·3
	7	11	1·4	14	1·6	11	1·4
	6	12	1·4	17	1·7	13	1·5
	5	11	1·4	15	1·6	13	1·5

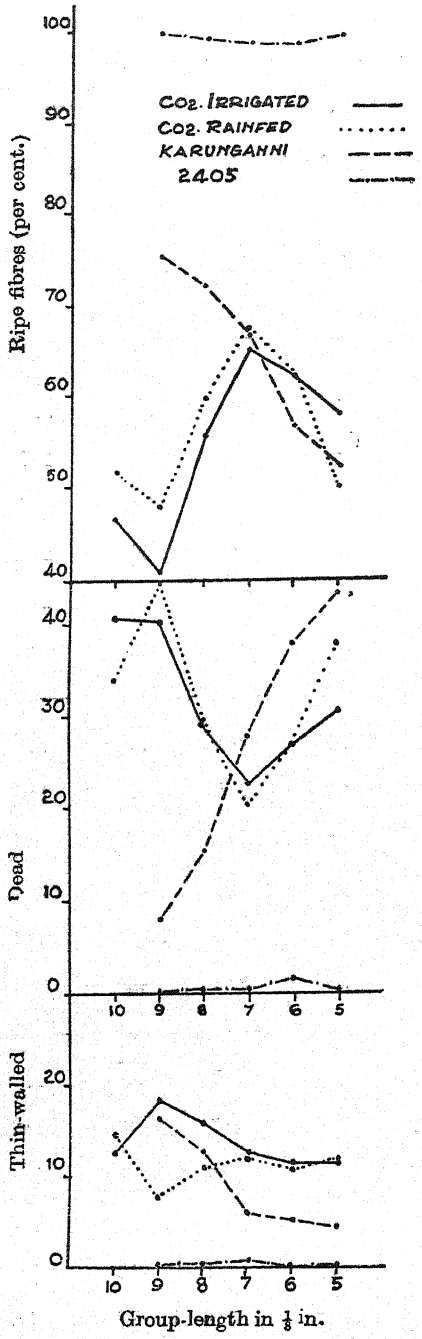
Co2. IRRIGATED
Co2. RAINFED
KARUNGANNI 2405
Ripe fibres (per cent.)
Dead
Thin-walled
Group-length in 1/8 in.

FIG. 2

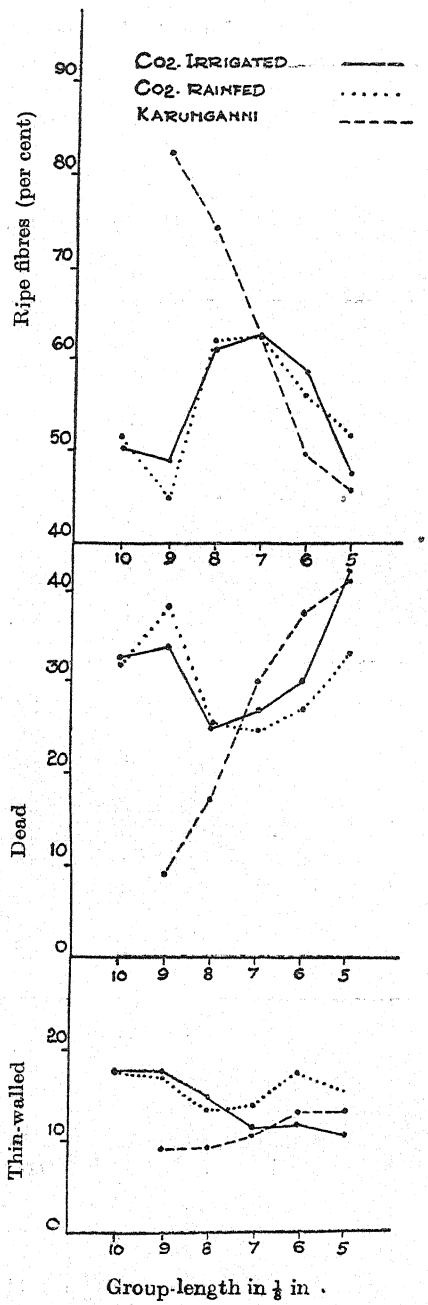
Co2. IRRIGATED
Co2. RAINFED
KARUNGANNI
Ripe fibres (per cent)
Dead
Thin-walled
Group-length in 1/8 in.

FIG. 3

(b) Convolutions

The frequency distribution of this character is recorded in Appendix I. It will be seen that the distribution is considerably skew excepting in the strain 2405 where it is normal. In the first three cottons the frequency in the first class-interval is so high that it makes the frequency polygon look like a combination of a normal curve with an exponential curve (Fig. 4 *a*). If the ripe fibres alone are considered, the distribution is normal (Appendix II).

The mean values of the number of convolutions per centimeter are given in Table II, column 6. As the number of fibres tested is considerably less than required for the correct estimation of this property, and as the observed distribution is very skew in all samples but one, the conclusions drawn should be considered to be only very approximate.

In the irrigated sample of Co 2 the number of convolutions per centimeter increase from 25·4 for the longest group-length to 50·6 for the 7/8 in. with a fall to 40·6 for the 5/8 in. In the same strain grown under rain-fed conditions the variation is not so large. It can be said to be constant in all the group-lengths at about 45 except for a rise to 59·8 in the 7/8 in. which group-length, it is interesting to note, records the highest value in the irrigated sample also. In Karunganni on the other hand the variation is more distinct and is only in one direction. It is 38·8 for the longest length and 20·0 for the shortest, the fall appearing to be gradual in the intermediate lengths. In the strain 2405 no regular trend of variation is apparent though some differences are significant.

The relationship between the convolutions and the maturity of the fibre may now be considered employing the ratio of fibre-diameter to wall-thickness as a measure of the maturity. The correlation coefficient cannot be calculated by the product-moment method as both the characters are not normally distributed. The modifications suggested by Yule [1929], namely, plotting the mean value of one of the characters corresponding to each group-interval of the other has been employed as shown in Fig. 5.

It will be seen that the regression lines are not linear, but appear to be portions of a rectangular hyperbola. The number of convolutions decreases with the decrease in the maturity. The ripe fibres (limits of the ratio 2·0 to 3·0) contain the largest number of convolutions, the thin-walled fibres (3·0 to 4·0) contain a lesser number and the dead fibres (above 4·0) the least.

It therefore follows that in the convolutions frequency-distribution curve (Fig. 4*a*) the portion corresponding to the small number of convolutions belongs to the immature fibres and that to the larger number to the ripe fibres; that is, the ' exponential ' part of the curve belongs to the immature and the ' normal ' part to the ripe fibres. This finding is in agreement with the statements of Denham [1923], Clegg [1932] and Gulati and Ahmad [1935].

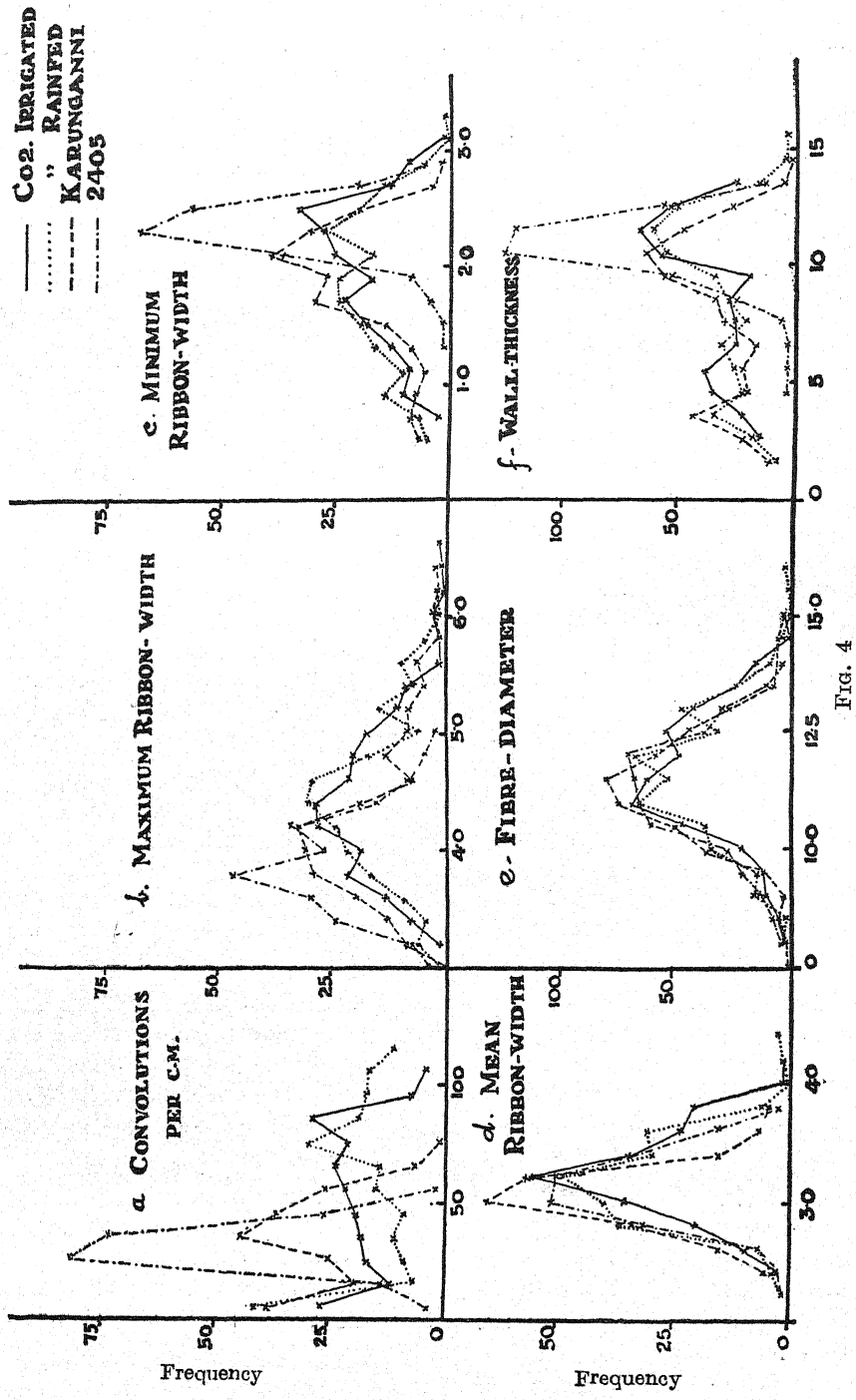
Co2. Irrigated
" Rainfed
Karunganni
2405
a Convolutions per c.m.
b. Maximum Ribbon-width
c. Minimum Ribbon-width
d. Mean Ribbon-width
e- Fibre-diameter
f- Wall thickness
Frequency
Frequency

Fig. 4

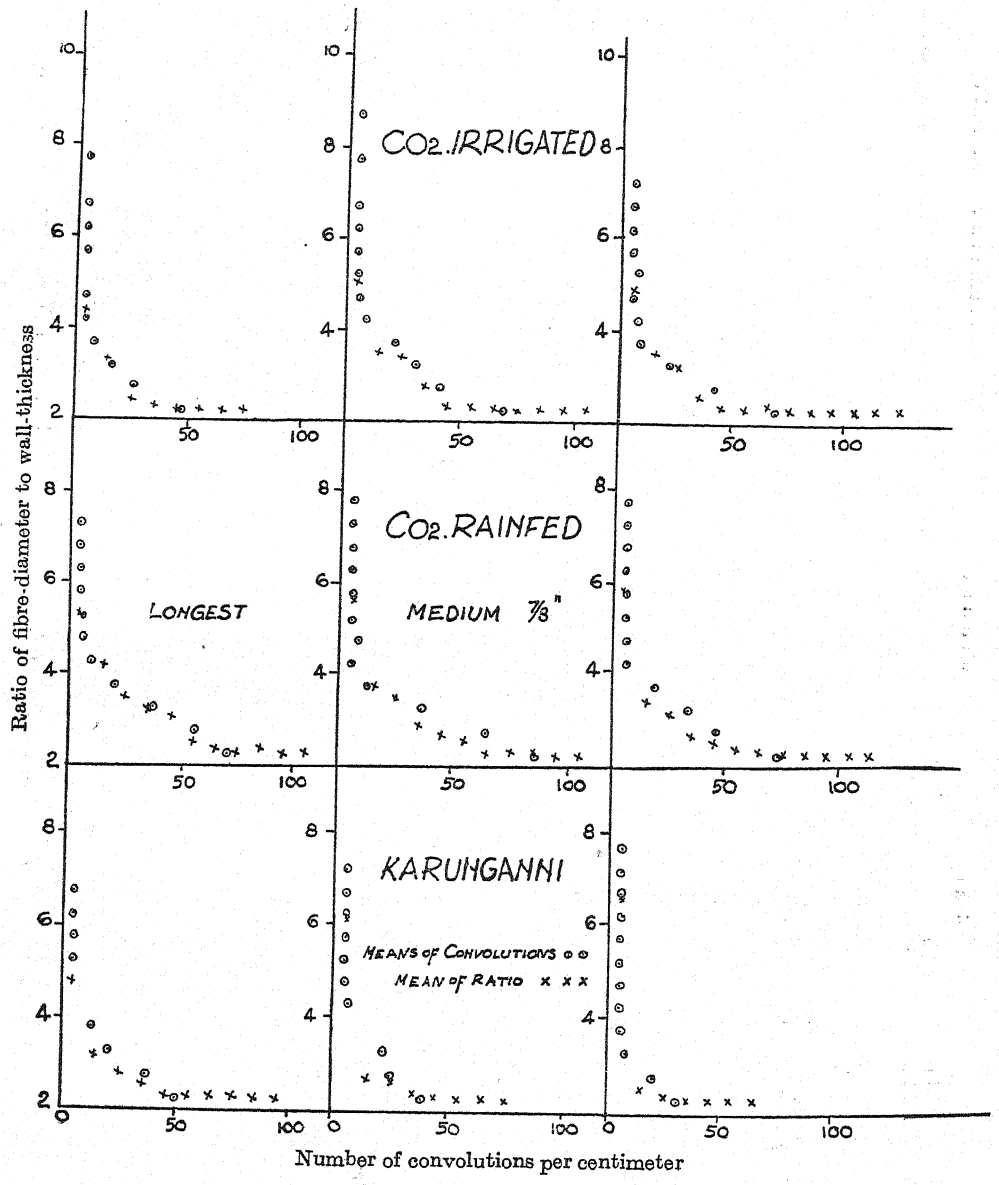
CO2. IRRIGATED
CO2. RAINFED
LONGEST
MEDIUM 7/8"
KARUNGANNI
MEANS of CONVOLUTIONS o o
MEAN of RATIO x x x
Ratio of fibre-diameter to wall-thickness
Number of convolutions per centimeter

FIG. 5

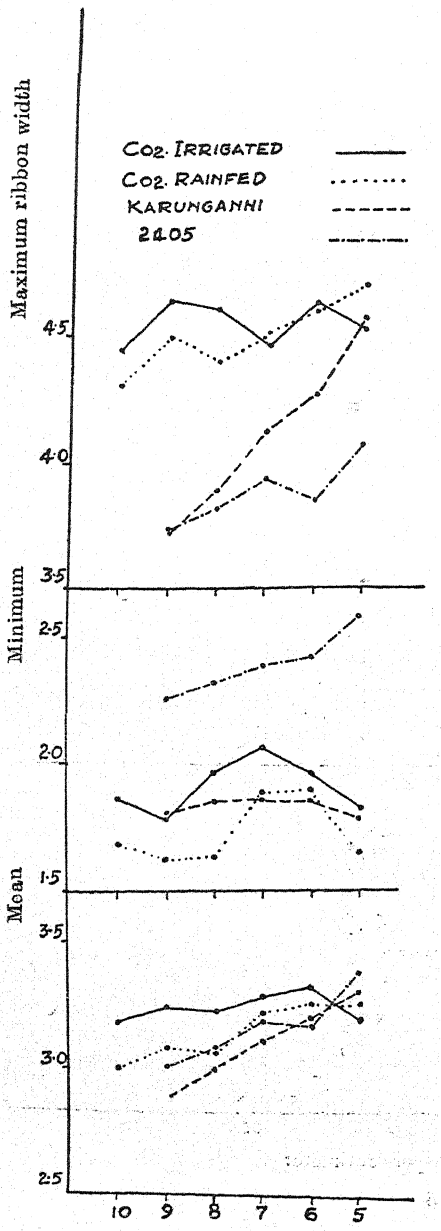

FIG. 6

(c) *Maximum ribbon-width*

The frequency distribution of this character is given in fig. 4*b**. For practical purposes the distribution can be taken as normal, though in some cases where the immaturity is large there is a tendency for the curve to get tailed at the farther end. It is obvious that the collapsed immature fibre, exhibiting a large ribbon-width, is responsible for this tailing. The distribution obtained by Koshal and Turner [1930] for this property is also normal. The mean values are given in Table II, col. 9.

In the rain-fed sample of Co 2 the variation is regular, *viz.*, rising steadily with the decrease of length. In the same strain grown under irrigation, though some of the values are significantly different from the others the regularity of the variation is not so apparent. Comparing the mean values for the irrigated with the rain-fed sample, it will be seen that irrigation appears to increase the maximum ribbon-width in the longer lengths (Fig. 6). In Karunganni the variation is more pronounced. Most of the differences are large and all of them significant and the value increases rapidly with the decrease in length. In strain 2405 the trend of variation is similar (except for the 6/8-in. group-length) though of a lower degree.

* For want of space the frequency distributions for all the group-lengths are not given in the paper. The frequencies for the one inch group-length are presented in Fig. 4, that length being the modal group-length for three out of the four cottons studied.

(*d*) *Minimum ribbon-width*

The distribution appears to depart from normality in Co 2 and Karunganni; the curve is much flattened and has often a tendency to become bi-modal in these strains as can be seen in Fig. 4*c*. This behaviour is again due to the presence of immature fibres. These fibres on account of their being thin, crowd about the smaller values and when the immaturity is large there is an inflation at this part of the curve, tending to make it depart from normality, and if the immaturity is sufficiently large this inflation makes the curve even bi-modal. All the group-lengths of Co 2 and the shorter ones of Karunganni record such distributions. The distribution tends to approach normality in the longer lengths of Karunganni probably on account of the small percentage of immature fibres in these lengths. In strain 2405, where there is practically negligible immaturity, the distribution is normal. The writer is not aware of any previous record of the distribution of this character.

The significances of the differences between the mean values can be assessed by the use of the standard error in strain 2405 only. The mean values are given in Table II, col. 11.

It will be seen that both in Co 2 and in Karunganni the differences are small and there is no apparent trend of variation. In strain 2405 the differences are all significant and the trend is distinct, *viz.*, increasing with the decrease of length (Fig. 6).

Irrigation appears to increase the value of the minimum ribbon-width especially in the longer lengths, as was found in the case of the maximum ribbon-width.

(*e*) *Mean ribbon-width*

This attribute is calculated along the lines described by Barritt [1930] as the mean of the maximum and minimum ribbon-widths. The distribution appears to be normal in all cases (Fig. 4*d*) and the standard error is very small when compared with that of the maximum ribbon-width. The immature fibres do not affect this property as they do the other two ribbon-widths. The mean values are recorded in Table II, col. 13.

In Co 2 irrigated some differences are statistically significant for odds 20 : 1 and there is a faint tendency for the mean ribbon-width to increase with the decrease of length (Fig. 6). In the rain-fed sample of the same strain the same tendency is more apparent. In Karunganni the trend of variation is the same and all the differences are significant. In strain 2405 also the behaviour is identical. Hence it can be stated that the general tendency is for the mean ribbon-width to rise steadily with the decrease of length.

The effect of irrigation appears to be to increase the mean ribbon-width, more especially in the longer lengths.

The values of the correlation coefficient between mean ribbon-width and fibre length, when all the fibres in all the group-lengths are taken together, has been worked out and these are given in Table IV.

TABLE IV

Cotton	Value of coefficient	N	Value for significance		Remarks
			$P=0\cdot5$	$P=0\cdot1$	
Co 2 irrigated . .	−0·066	1200	0·0577	0·0750	Significant
Co 2 rain-fed . .	−0·274	1200	0·0577	0·0750	Highly significant
Karunganni . .	−0·479	1000	0·0632	0·0822	Highly significant
Strain 2405 . .	−0·346	1000	0·0632	0·0822	Highly significant

It is seen that all the coefficients are negative and significant.

Taking the mean ribbon-width as a measure of the fineness of cotton, it can therefore be stated that the fineness improves with increase of length. According to Balls [1928] the "general tendency to correlation between length and fineness throughout genus *Gossypium*" is also the same. The association of fineness with length thus appears to be a general rule.

In discussing the usefulness of the mean ribbon-width Barritt [1930] states "the two extreme measurements cause considerable increase in the dispersion due to the immature hairs, so that the standard deviation of the mean becomes a useful measure of the degree of immaturity of the sample." The results obtained in the present investigation may incidentally be utilised to verify this conclusion. The results are recorded in Table V. The strain 2405 has been omitted as there is negligible amount of immaturity in this strain.

TABLE V

Group length in 1/8 in.	Co 2 irrigated			Co 2 rain-fed			Karunganni		
	S. E.	Ripe fibres (per cent)	Dead fibres (per cent)	S. E.	Ripe fibres (per cent)	Dead fibres (per cent)	S. E.	Ripe fibres (per cent)	Dead fibres (per cent)
10	0·020	46·5	40·8	0·020	51·5	34·0			
9	0·020	41·0	40·5	0·020	47·8	44·5	0·017	75·2	8·0
8	0·023	55·5	28·8	0·019	59·5	29·5	0·018	72·0	15·2
7	0·024	64·8	22·5	0·023	67·5	20·5	0·019	66·8	27·8
6	0·031	62·0	26·5	0·028	61·8	27·2	0·021	56·5	38·0
5	0·030	57·7	30·5	0·031	50·0	37·8	0·020	52·2	43·5

The results indicate that in both the samples of Co 2 the standard error increases steadily with the fall in length in spite of the significant variations of the immaturity in nearly the opposite direction. In Karunganni, though there is a large rise in the immaturity with the decrease in length, the variation of the standard error is very little. It is much smaller than that found even in the strain Co 2, where the variation in the maturity is less marked. Hence the conclusion of Barritt, namely, that standard error of the mean ribbon-width being a measure of the maturity, cannot be said to be borne out in the samples under study.

(*f*) *Swollen fibre-diameter*

The diameter measured in the present enquiry corresponds to the swollen diameter studied by Calvert and Summers [1925] and by Barritt [1929] with this difference, namely, that in the present case the fibre is held tight at the two ends so that it is not allowed to contract during the swelling process. The frequency distributions are given in Fig. 4*e*. It may be recalled that the number of fibres tested is 400 in the first three cottons and 200 in the fourth, namely, strain 2405. The mean values are recorded in Table II, col. 16.

In the strain Co 2, both irrigated and rain-fed, the fibre-diameter increases steadily with the decrease in length up to 6/8 in. with a slight fall in the 5/8 in. (Fig. 7). Most of the differences, even between consecutive group-lengths, are significant. In Karunganni also the diameter increases with the fall in length; the difference between 6/8 in. and 5/8 in. alone is not, however, statistically significant. In strain 2405 also the same trend of variation is noticed and all the differences are significant. The increase of fibre-diameter with the decrease in length appears thus to be the general rule.

The effect of irrigation in the strain Co 2 appears to be to increase the diameter in the longer lengths, as was found to be the tendency in the case of the ribbon-widths, indicating that the fibre-cells tend to grow bigger under irrigation.

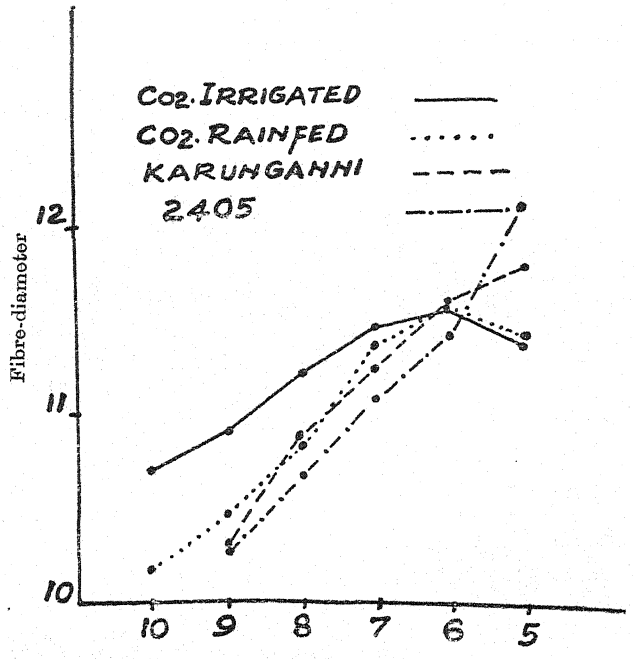

FIG. 7

(g) Wall-thickness

The distribution of this character (Fig. 4*f*) is similar to that of the minimum ribbon-width. The mean values are given in Table II, col. 18.

In the strain Co 2 and Karunganni the variations do not exhibit any definite trend (Fig. 8). In strain 2405, however, the wall-thickness increases steadily with the decrease in length and all the differences are significant.

(h) Weight of dead and thin-walled fibres

From the mean values of the ratio $D/D—d$ for the different categories of fibres, it is possible to determine the weights of the thin-walled and dead fibres in terms of the fully ripe fibre. The volume of cellulose in the fibre having a diameter, D, and internal canal of diameter, d, is equal to $\frac{\pi}{4}(D^2—d^2)$ per unit length. The volume of a fully ripe fibre of the same diameter is $\frac{\pi}{4} D^2$ for the same length. Assuming the density of the cellulose to be the same in both *, the ratio of the weights of the two is given by $\frac{D^2—d^2}{D^2}$

* This assumption may not be perfectly valid because the swollen cellulose is under pressure inside a ripe fibre while there is not so much pressure inside an immature fibre, there being more available space for expansion inside it. Due to the higher pressure the density may be a little more in the ripe fibre.

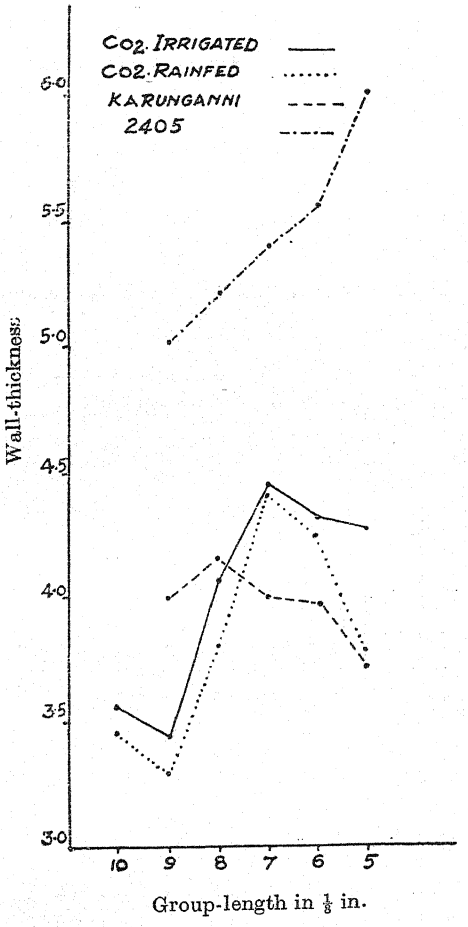

FIG. 8

The value of the ratio $D/D—d$ for the ripe fibres ranges from 1·0 to 1·5; most of the values, however, lie in the neighbourhood of 1·0 and the mean value comes to less than 1·1. Calculating from this value the weight of a fibre in the 'ripe' class comes to 0·992 of a perfectly ripe fibre, whose weight is taken as 1·0. For the thin-walled fibres the ratio ranges from 1·5 to 2·0 and the mean can be taken as 1·75. This gives the weight of the thin-walled fibre as 0·816 of the perfectly ripe. For the dead fibres the mean value of the ratio varies rather widely, ranging from 2·5 to 5·0 or even more in a few cases. But for most of them the mean can be taken as 3·3 which gives the weight as 0·487.

The values got by Peirce and Lord [1934] by actual weighing are 0·75 and 0·45 respectively for the thin-walled and dead fibres. The figures obtained by them from microscopical measurements also are nearly the same, being 0·69 and 0·74 for the thin-walled and 0·38 for the dead. The results obtained in the present case should, therefore, be considered to be a little higher. This might be due to the choice of the smaller upper limit (4 and not 5, as according to these authors) for the thin-walled fibres in the present case as already referred to on page 311.

V. Conclusions

The following conclusions can be drawn from the results considered in the foregoing :—

(1) The variation of the maturity of fibres with respect to length is different in different strains ; further work is to be done before it is possible to state that a particular variation is due to strain or culture.

(2) The convolutions per unit length also indicate different trends of variation in the different cottons.

(3) The maximum ribbon-width, mean ribbon-width and swollen diameter generally decrease with the increase of length, indicating the general tendency of improvement of fineness with length.

(4) The minimum ribbon-width and swollen wall-thickness are not reliable measures on account of their skew distributions in three out of four cottons studied in the present work.

(5) The number of convolutions per unit length has a definite relationship with the maturity of the fibre, decreasing with the decrease in the degree of maturity.

(6) A general survey of the standard errors discloses the fact that fibres are more uniform in their physical properties in the longer lengths than in the shorter.

(7) The effect of irrigation in strain Co 2 appears to be to increase the diameter of the fibre-cell, more especially in the longer lengths.

(8) The mean weights of the thin-walled and dead fibres, calculated from the respective mean values of the ratio of swollen fibre-diameter to swollen wall-thickness, are found to be slightly greater than the values obtained by Peirce and Lord.

VI. Acknowledgments

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APPENDIX I

Convolutions per cm. (all fibres)

Class interval	Co 2 irrigated						Co 2 rain-fed						Karunganni					Class interval	Strain 2405				
	10/8	9/8	8/8	7/8	6/8	5/8	10/8	9/8	8/8	7/8	6/8	5/8	9/8	8/8	7/8	6/8	5/8		9/8	8/8	7/8	6/8	5/8
0—10	82	69	50	27	56	67	59	80	69	41	65	85	22	29	39	55	84	0—5	...	2	3	4	1
11—20	16	23	9	12	8	9	13	3	12	6	13	7	22	18	19	19	20	6—10	3	0	0	2	1
21—30	12	17	14	17	7	12	6	5	7	8	6	10	24	39	25	28	32	11—15	9	4	3	4	5
31—40	26	20	11	18	11	8	9	10	7	11	10	9	33	33	45	50	34	16—20	29	7	10	19	17
41—50	28	21	23	19	19	13	13	9	11	8	13	9	33	38	37	35	27	21—25	46	28	30	34	32
51—60	23	14	29	22	12	18	23	15	8	15	12	15	34	32	26	10	3	26—30	58	37	52	50	50
61—70	9	14	24	24	19	12	26	13	17	14	23	15	22	10	6	3	...	31—35	37	47	41	51	46
71—80	4	13	19	21	30	23	19	18	18	30	23	17	8	1	1	...	...	36—40	17	48	33	19	26
81—90	...	7	12	29	25	16	20	12	17	19	19	12	1	...	...	...	...	41—45	1	21	16	13	16
91—100	...	2	7	7	6	7	7	15	17	17	8	8	1	...	...	...	...	46—50	...	4	10	3	3
101—110	...	...	2	4	5	3	2	10	4	17	5	6	...	...	...	...	...	51—55	...	2	2	0	3
111—120	...	...	...	...	2	1	3	8	6	11	2	2	...	...	...	...	...	56—60	...	...	...	0	...
121—130	...	...	...	...	...	1	...	2	6	0	1	3	...	...	...	...	...	61—65	...	...	...	0	...
131—140	...	...	...	...	...	...	...	...	0	1	...	2	...	...	...	...	...	66—70	...	...	...	0	...
141—150	...	...	...	...	...	...	...	...	1	2	...	...	...	...	...	...	...	71—75	...	...	...	1	...

APPENDIX II

Convolutions per cm. (ripe fibres only)

Class interval	Co 2 irrigated						Co 2 rain-fed						Karunganni					Class interval	Strain 2405				
	10/8	9 8	8/8	7/8	6/8	5/8	10/8	9/8	8/8	7/8	6/8	5/8	9/8	8/8	7/8	6/8	5/8		9/8	8/8	7/8	6/8	5/8
0—10 . . .	1	...	...	1	1	2	...	...	1	2	1	1	...	1	1	...	3	0—5 .	...	...	...	1	...
11—20 . . .	6	...	1	3	3	2	...	1	0	0	3	2	9	10	15	12	18	6—10 .	2	...	...	2	1
21—30 . . .	11	4	1	3	4	3	...	2	1	1	3	5	14	28	19	26	32	11—15 .	9	4	3	4	5
31—40 . . .	25	10	5	14	6	7	2	10	2	6	9	6	29	31	46	50	34	16—20 .	29	7	10	19	17
41—50 . . .	28	20	18	18	14	13	5	7	7	6	13	7	33	38	37	35	27	21—25 .	46	28	30	34	32
51—60 . . .	23	13	27	21	11	18	20	15	8	14	11	12	34	32	26	10	3	26—30 .	58	37	52	50	50
61—70 . . .	9	14	23	23	19	21	25	13	17	14	23	15	22	10	6	3	...	31—35 .	37	47	41	51	46
71—80 . . .	4	13	19	21	30	23	19	18	18	29	24	17	8	1	1	...	...	36—40 .	17	48	3	19	26
81—90 . . .	...	7	12	29	25	16	19	12	16	18	19	12	1	...	...	...	...	41—45 .	1	21	16	13	16
91—100 . . .	...	2	7	7	6	7	7	15	17	17	8	8	1	...	...	...	...	46—50 .	...	4	10	3	3
101—110 . .	...	...	2	4	5	3	2	10	4	17	5	6	...	...	...	...	...	51—55 .	...	2	2	0	3
111—120 . . .	...	...	...	...	2	1	3	8	6	11	2	2	...	...	...	...	...	56—60 .	...	...	...	0	...
121—130 . . .	...	...	...	...	...	1	...	2	6	0	1	3	...	...	...	...	...	61—65 .	...	...	...	0	...
131—140 . . .	...	...	...	...	...	...	...	...	0	1	...	2	...	...	...	...	...	66—70 .	...	...	...	0	...
141—150 . . .	...	...	...	...	...	...	...	...	1	2	...	...	...	...	...	...	...	71—75 .	...	...	...	1	...

STUDIES ON THE ROOT-ROT DISEASE OF COTTON IN THE PUNJAB

VI. CHEMICAL COMPOSITION OF HEALTHY AND DISEASED COTTON PLANTS

BY

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I. Introductory

The present investigation is part of an extensive work which is being carried out in this Laboratory on the pathological aspect of the root-rot disease of cotton in order to discover control measures for this serious disease. The work includes quantitative study of changes that occur in the plants that have died as a result of the root-rot disease.

Plants that have recently wilted on account of the attack of root-rot have all their root tissues, particularly the bark, broken down into shreds and can be easily pulled out of the ground. The disease actually starts much earlier and the manifestation of the disease above ground in the form of wilt is a very late symptom. Roots of diseased plants are moist and sticky to the touch, and leave a yellow stain on white surface [Vasudeva, 1935].

The disease invariably appears in patches, but sometimes when two plants are growing at the same spot only one of them might succumb to root-rot. The work reported in the present paper was undertaken with a view to determine the differences in the chemical composition of the diseased and healthy cotton plants growing in the same field.

II. Material and method

Sampling was carried out in plot No. 46/24 of the Botanical area at the Agricultural Research Station, Lyallpur. Samples of healthy and diseased cotton plants of variety 15-Mollisoni standing side by side in the same field were collected for analysis. Plants which had just begun to show the first signs of wilting, *i.e.*, the wilting of the topmost leaves, represented the diseased samples. As far as possible care was taken to select plants of the same age, size and height. The plants were dug out very carefully so that the greater part of the root-system was left intact. The digging, in order to expose the lowermost portion of roots, had to be carried out up to a depth of four feet. As soon as the entire plant was pulled out, it was divided into root, stem and leaf portions and each part was immediately put into separate air-tight glass jars. About thirty to fifty plants of each sort, *i.e.*, diseased and healthy, were worked into a composite sample which formed the final representative sample. Sampling was carried out throughout the season between 8 and 10 A.M. As soon as the samples were taken, they were carefully washed in a gentle spray of running water in order to remove the adhering soil particles. Care was always taken to prevent leaching during the cleaning operation.

The washed samples were carefully dried between folds of filter paper.

A. Sample for carbohydrate analysis

A portion of the fresh sample of root, stem and leaf of diseased and healthy plants was weighed and put into 90 per cent boiling alcohol. After a few minutes' boiling the samples were kept in air-tight jars with a few drops of toluene for further use.

The remaining material was put in hot air oven at 95°C. where it was left for about half an hour in order to stop the enzymic activity. The temperature of the oven was then brought down to about 50 to 55°C. The sample was left to dry at this temperature till constant weight was obtained. After the sample had dried, it was ground down to pass a 100-mesh sieve. The sample thus prepared was preserved in air-tight bottles and this formed the starting material for all subsequent analyses.

B. Sample for chemical analysis

The dried material was ashed in 500 c.c. porcelain dishes over the open Bunsen burner flame. The material was kept stirring with a glass rod in order to prevent fusion of the ash ingredients. The temperature of the ash under the conditions was not allowed to rise above 500°C. When almost constant weight of ash was obtained, it was preserved in air-tight bottles for further use. For the chemical analysis, a known weight of the ash was extracted with concentrated hydrochloric acid for eight hours as detailed in the Official Method [1936].

The extract thus prepared was evaporated to complete dryness over water-bath, where it was left for four to five hours to dehydrate silica. After dehydration of silica, the residue was taken up with dilute hydrochloric acid, made up to a known volume and filtered. The residue on the filter paper was washed, dried and ignited for the determination of the soluble silica content of the ash in the filtrate. The various mineral constituents were determined by methods described elsewhere.

III. Experimental.

A. Carbohydrates

The carbohydrates in the preserved samples were extracted with 80 per cent boiling alcohol using Soxhlet's extraction apparatus. The extraction was always allowed to proceed overnight. The extract was evaporated almost to dryness at 40°C. and the residue was taken up with distilled water. The gummy matter in the water extract was precipitated with lead acetate and the excess of lead acetate was removed by adding to the filtrate an extra quantity of sodium phosphate and filtering again. A few drops of toluene were added to the filtrate as a preservative. Reducing sugars and total sugars were estimated separately, the difference being reckoned as sucrose. The method followed for the determination of reducing and total sugars was essentially the same as described by Hanes [1929] and later by Hulme and Narain [1931]. The method consists in oxidising the reducing substances with excess of potassium ferricyanide. The excess of the reagent is made to liberate an equivalent amount of iodine from potassium iodide, which is titrated with standard thiosulphate solution.

The results are calculated on the basis of the fresh weight of the material taken. The results of the analyses are set out in Table I.

TABLE I

Distribution of carbohydrates in healthy and diseased cotton plants (mg. in 100 grams of fresh material)

Date of sampling	Material	Reducing sugars			Total sugars			Sucrose		
		H	D	*t*	H	D	*t*	H	D	*t*
19th June 1937	Root	303·0	674·0	10·9	1918·0	2725·0	2·5	1615·0	2051·0	3·4
29th June 1937		219·5	669·0		2106·0	3328·0		1986·5	2659·0	
13th July 1937		239·0	297·4		1168·0	1274·0		929·0	976·6	
27th July 1937		154·0	340·0		778·3	742·6		624·3	402·6	
13th Aug. 1937		113·3	191·0		495·4	1044·0		382·1	853·0	
19th June 1937	Stem	478·0	761·0	4·5	1345·0	2230·0	5·8	867·0	1469·0	5·7
29th June 1937		587·6	1130·6		1417·0	2690·0		829·4	1559·4	
13th July 1937		313·3	510·0		814·0	1505·0		500·7	995·0	
27th July 1937		285·0	424·0		778·3	...		493·3	...	
13th Aug. 1937		302·5	748·0		1009·0	2530·0		706·5	1782·0	
19th June 1937	Leaf	306·2	506·2	6·1	1239·0	1629·0	4·7	932·8	1122·8	2·8
29th June 1937		318·6	729·0		1060·0	1770·0		741·4	1041·0	
13th July 1937		283·2	789·1		920·1	1911·0		636·9	1121·9	
27th July 1937		347·0	644·0		566·3	955·6		219·3	311·6	
13th Aug. 1937		447·0	708·0		1115·0	2265·0		668·0	1557·0	

H=Healthy, D=Diseased.

The statistical significance of the differences in healthy and diseased plants was evaluated by ' Student's Method ' as described by Fisher [1925]. In this method a function, *t*, is calculated from the data and the probability of the result recorded being significant is greater or less according as this number is large or small. For a series of about five observations the value of '*t*'=2·57 is on the margin of significance (the usual 20 : 1 standard being adopted).

The value of '*t*' for comparison of reducing sugars is fairly high, showing thereby that the results recorded are significant and that the reducing sugars in all parts of diseased plants, *i.e.*, root, stem and leaf are higher than in the respective parts of the healthy plants. The total sugars are significantly high both in the stem and leaf of the diseased plants. In the case of diseased roots the total sugars are high in four samples, but the results are negative in one sample.

Sucrose is also higher in the root, stem and leaf of the diseased plants.

B. NITROGEN

Nitrogen in the diseased and healthy plants was estimated as total nitrogen, nitrate nitrogen and ammoniacal nitrogen.

Total nitrogen was determined by the Official Gunning method [1936] modified to include the nitrogen of nitrates.

The inorganic fraction of nitrogen in the plant material was determined by the method described by Session and Shive [1928].

The method followed consists in distilling over the ammonia formed with a strong current of air. The free ammoniacal nitrogen in the plant material is first liberated with saturated sodium carbonate solution containing dissolved sodium chloride. The ammonia liberated is distilled over into standard acid contained in wide-mouthed glass tubes connected with the reaction flasks. A strong current of air is allowed to pass overnight both through the reaction flasks and the receivers. The system is disconnected and the excess acid is titrated against standard alkali. To the reaction flask is now added a known weight of Devarda's alloy and enough sodium hydroxide to give approximately $N/8$ solution in the reaction flask, from which acid is poured into the receiver tubes and a few drops of methyl red indicator added. Aeration is again allowed to proceed overnight and the excess acid is titrated against standard alkali. In this method there is no likelihood of any other portion of nitrogen, except the nitrate, coming over, as the entire experiment is conducted in the cold.

The results of the nitrogen estimations in different parts of healthy and diseased plants are given in Table II.

TABLE II

Distribution of nitrogen in healthy and diseased cotton plants (mg. per 100 gram dry matter)

Date of sampling	Material	NH_3—nitrogen			NO_3—nitrogen			Total nitrogen		
		H	D	t	H	D	t	H	D	t
19th June 1937	Root	10·0	19·0	2·9	214·0	186·0	5·2	1270·0	1630·0	11·8
29th June 1937		4·6	7·4		163·0	150·0		970·0	1390·0	
13th July 1937		...	...		...	...		1610·0	2070·0	
27th July 1937		3·2	6·5		170·0	136·0		1110·0	1380·0	
13th Aug. 1937		6·0	8·0		83·0	63·0		1180·0	1580·0	
19th June 1937	Stem	14·0	17·0	3·9	672·0	479·0	4·8	2210·0	2070·0	0·4
29th June 1937		14·0	20·0		409·0	357·0		1790·0	1820·0	
13th July 1937		4·2	13·5		425·0	234·0		2230·0	1940·0	
27th July 1937		8·3	17·7		414·0	289·0		1800·0	2000·0	
13th Aug. 1937		7·9	9·7		157·0	123·0		...	...	
19th June 1937	Leaf	42·0	48·0	3·4	108·0	134·0	2·7	4700·0	4440·0	1·5
29th June 1937		37·0	39·0		117·0	144·0		3820·0	3990·0	
13th July 1937		52·0	63·0		139·0	162·0		4570·0	4210·0	
27th July 1937		42·0	50·0		151·0	169·0		4220·0	4260·0	
13th Aug. 1937		29·0	31·0		111·0	103·0		4960·0	3520·0	

The following are the chief points of interest :—

(*a*) Total nitrogen in the diseased roots is significantly higher than in healthy roots.

(*b*) Ammoniacal nitrogen is significantly higher in roots, stems and leaves of the diseased plants.

(*c*) Nitrate nitrogen is significantly higher in roots and stems of healthy plants.

C. Chemical analysis

(*a*) *Estimation of ash, iron and phosphorus*

Ash.—This was determined according to the method already described.

Iron.—In samples of the hydrochloric acid extract of the plant material iron was estimated colorimetrically by Daniel and Harper's micro-method [1934]. This method consists in oxidising the iron into ferric state with a few drops of concentrated nitric acid and subsequently developing the colour with potassium sulphocyanide and comparing the tinge with controls containing known quantitities of iron. Standard iron solution was prepared by dissolving known weight of reagent grade ferrous ammonium sulphate in distilled water and oxidising with potassium permanganate. It was found during the course of these estimations that if either hydrochloric acid or nitric acid was omitted from the standard solution the shade of the colour developed with sodium sulphocyanide would not compare with that of the test solution. Accordingly the same quantities of hydrochloric acid and nitric acid were added to the control solution as were contained in the test solution.

Phosphorus.—Phosphorus was determined colorimetrically by the Official micro-method [1936]. The colour is developed with ammonium molybdate solution. Hydroquinine solution is then added to the test sample, the latter in the presence of sodium sulphite develops a blue colour with the solution containing phosphate. The tinge was compared with controls containing known amounts of phosphate.

The results of ash, iron and phosphorus estimations are given in Table III.

TABLE III

Per cent ash, iron and phosphorus in healthy and diseased cotton plants

Date of sampling	Material	Ash (grm. per 100 grm. dry matter)			Iron (mg. per 100 grm. dry matter)			Phosphorus (mg. per 100 grm. dry matter)		
		H	D	*t*	H	D	*t*	H	D	*t*
19th June 1937	Root	6·92	6·20	1·8	25·9	29·7	2·7	206·8	183·8	1·2
29th June 1937		5·33	5·84		17·3	23·9		45·9	79·6	
13th July 1937		8·71	6·64		38·2	40·6		121·4	112·8	
27th July 1937		5·75	5·07		21·3	28·6		143·1	127·0	
13th Aug. 1937		4·45	3·62		32·6	32·2		161·4	157·4	
19th June 1937	Stem	11·20	9·09	4·7	15·0	17·9	1·5	290·0	239·4	0·7
29th June 1937		8·62	7·42		11·5	10·6		111·0	123·0	
13th July 1937		9·42	6·98		19·3	16·0		126·5	114·1	
27th July 1937		8·30	6·59		21·7	16·1		152·5	142·0	
13th Aug. 1937		5·39	4·89		24·7	12·1		93·9	113·0	
19th June 1937	Leaf	20·40	17·70	2·0	43·1	27·9	1·8	378·0	360·0	0·2
29th June 1937		19·85	19·26		40·9	37·8		219·0	256·0	
13th July 1937		14·67	15·26		27·7	32·3		295·0	289·0	
27th July 1937		16·49	15·48		38·5	29·2		356·0	367·7	
13th Aug. 1937		16·82	13·96		33·0	24·6		252·0	238·0	

From the data the general conclusions deduced are as follows :—

(*i*) There is no significant difference in the ash of diseased and healthy roots and leaves.

Stems of diseased plants contain lower percentage of ash.

(*ii*) The diseased roots contain a higher amount of iron and the results are statistically significant.

The amount of iron in diseased samples of stems and leaves is lower in four out of five samples analysed, but the results are not significant.

(*iii*) There is no significant difference in phosphorus content of roots, stems and leaves of healthy and diseased plants.

(*b*) *Estimation of potassium, sodium and calcium*

Potassium.—Determinations of potassium were made according to the method described by Milne [1929]. Potassium was precipitated with cobaltinitrite reagent.

Sodium.—This was determined gravimetrically by precipitation with Kahn's reagent, *i.e.*, magnesium uranyl acetate [1930]. The precipitate was washed with alcohol and ether and weighed. Before the actual estimation of sodium and potassium, the test solutions were completely freed from extraneous elements. Throughout these estimations a reagent control was run side by side.

Calcium.—This was estimated in the hydrochloric acid extract microchemically by the Official method. Calcium was precipitated as oxalate at *p*H 5.

The results of potassium, sodium and calcium estimations are given in Table IV.

TABLE IV

Per cent potassium, sodium and calcium in healthy and diseased cotton plants (mg. per 100 grms. dry matter)

Date of sampling	Material	Calcium			Sodium			Potassium			Ca/K ratio		
		H	D	*t*	H	D	*t*	H	D	*t*	H	D	*t*
19th June 1937	Root	320·0	368·0		...	...		...	...		...		
29th June 1937		282·4	356·1		86·0	37·0		3644·0	2996·0		0·078	0·120	
13th July 1937		415·0	482·0	2·5	71·0	96·0	0·1	3677·0	2390·0	2·9	0·113	0·200	3·4
27th July 1937		327·0	334·0		44·0	65·0		1984·0	1818·0		0·165	0·184	
13th August 1937		351·0	349·0		40·0	35·0		1784·0	1203·0		0·197	0·290	
19th June 1937	Stem	1550·0	1250·0		86·0	85·0		1926·0	1420·0		0·805	0·880	
29th June 1937		1194·0	1108·0		77·3	53·0		2258·0	1910·0		0·529	0·580	
13th July 1937		2460·0	2410·0	2·3	69·0	41·0	3·0	3603·0	2350·0	1·4	0·683	1·020	0·0
27th July 1937		2533·0	1551·0		88·0	25·0		2631·0	1569·0		0·963	0·989	
13th August 1937		2155·0	1557·0		64·0	30·0		1801·0	2507·0		1·200	0·620	
19th June 1937	Leaf	5334·0	4440·0		88·0	79·0		1004·0	1021·0		5·310	4·350	
29th June 1937		5397·0	4845·0		112·0	114·0		3428·0	4307·0		1·570	1·120	
13th July 1937		3950·0	3690·0	5·1	75·0	104·0	0·3	2706·0	2672·0	1·1	1·460	1·380	2·4
27th July 1937		4170·0	3770·0		45·0	63·0		2108·0	2208·0		1·980	1·710	
13th August 1937		4210·0	3630·0		126·0	64·0		...	2606·0		...	...	

The data bring out the following points of interest.

(*i*) The roots of diseased plants contain less potassium.

(*ii*) The stems of diseased plants contain less sodium.

(*iii*) Diseased leaves contain significantly less calcium.

(*iv*) There are indications that the diseased roots contain a higher amount of calcium, results being on the margin of significance. The percentage of calcium in diseased plants as a whole is, however, significantly less.

(*v*) It is observed that calcium-potassium ratio is significantly higher in diseased roots.

(*c*) *Total sugars and potassium ratio*

The ratios deduced from the carbohydrate and potassium analyses are given in Table V.

TABLE V

Total sugars/potassium in healthy and diseased cotton plants

Sample	Material	Healthy	Diseased	*t*
1	Root	..	..	1·9
2		0·579	1·110	
3		0·318	0·533	
4		0·392	0·408	
5		0·278	0·868	
1	Stem	0·698	1·570	6·8
2		0·627	1·410	
3		0·226	0·640	
4		0·296	..	
5		0·560	1·010	
1	Leaf	1·230	1·600	4·4
2		0·309	0·411	
3		0·340	0·715	
4		0·269	0·433	
5		..	..	

It is clear from the data that total sugar/potassium is higher in stem and leaves of the diseased plants.

(d) Distribution of various elements in the leaf and root

Comparative distribution of iron, calcium and potassium in the leaf and root of healthy and diseased cotton plants is shown in Table VI.

TABLE VI

Comparative distribution of various elements in the leaf and root of healthy and diseased cotton plants

Sample	Fe in leaf/Fe in root			Ca in leaf/Ca in root			K in leaf/K in root		
	H	D	*t*	H	D	*t*	H	D	*t*
1	1·66	0·94		16·67	12·07		..	..	
2	2·36	1·58		19·11	13·61		0·94	1·44	
3	0·73	0·79	3·0	9·52	7·65	4·1	0·73	1·12	3·5
4	1·81	1·02		12·76	6.05		1·06	1·21	
5	1·01	0·76		12·0	10·4		..	2·17	

The results given above show that (*i*) the ratios of iron in leaf to iron in root and calcium in leaf to calcium in root are significantly lower in diseased plants. This means that although there appears to be accumulation of these two elements in the roots, the stems and leaves of the diseased plants are not able to make use of it. Further investigation must decide whether this condition is the result of disease or *vice versa*. (*ii*) the ratio of potassium in leaf to potassium in root is significantly higher in diseased plants.

Thanks are due to Professor J. C. Luthra, I.A.S., and Dr. P. E. Lander, I.A.S. for their keen interest throughout the course of this investigation. Thanks are also due to Dr. Ramji Narain for valuable suggestions and to the Indian Central Cotton Committee for financing the scheme.

The junior author carried out some of the analytical work under the guidance of the senior author.

IV. Summary

The diseased cotton plants which have wilted as a result of the root-rot attack exhibit marked differences in chemical composition as follows :—

1. Reducing sugars and sucrose are higher in all parts, *i.e.*, root, stem and leaf, of diseased than in the corresponding parts of healthy plants.
2. Total and ammoniacal nitrogen, iron and the ratio of calcium to potassium are significantly higher in diseased than in healthy roots.
3. The ratios of iron in leaf to iron in root and calcium in leaf to calcium in root are lower in diseased plants and the ratio of potassium in leaf to potassium in root is higher in diseased than in healthy plants.

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NOTE

DECREE No. 24000/1936 F. M. OF ROYAL HUNGARIAN MINISTRY OF AGRICULTURE ON THE SUBJECT OF GOVERNMENT PHYTOSANITARY SERVICE REGULATIONS

Section 1

By reason of Articles 1, 2 and 3 of Law XVII of 1936 on the subject of the insertion of the International Agreement of Rome of April 16th, 1929, on plant protection, and based on Section 1, para. 2 of Decree No. 6300/1936 M. E. concerning the cessation of the Ministry in the Trade of Medicinal and Industrial Plants, I have decided on the constitution of a Government Phytosanitary Service to prevent and diminish loss caused to Farm and garden produce, whether by harmful insects, parasitic plants, or biological change.

Section 2

The objects of the Government Phytosanitary Service are as under: Co-operation with civil authorities in the enforcement of decrees and laws already passed or any future laws on the subject of the prevention of, and struggle against, loss in Farm and garden produce caused either by harmful insects, parasitic plants, or biological change. Among these laws and decrees we quote; Art. XV of 1882 with reference to the insertion of the International Agreement of Berne of November 3rd, 1881, on the protective measures to be taken against " phylloxera vastatrix " as well as the complementary article of the law already mentioned. Art. XLI of 1889; Art. XVII of 1883 on the same subject; Art. XII of 1894 on Agriculture and Supervision of Fields; Executive clause No. 48,000 of 1894 of the preceding law; Art. XXXI of 1907 on the destruction of the Moroccan Locust and Executive Decree No. 28,000 of 1907 of the preceding article; Art. XLIV of 1925 on the precautions to be taken to prevent the arrival from abroad and spreading of potato diseases such as potato gangrene, and the Colorado Insect; Executive Clause No. 40,000 of 1926 of the preceding Article; Art. XVII of the 1936 law concerning the insertion of the international agreement of Rome of April 16th, 1929, on the protection of plants, the encouragement of administrative authorities to activity in that respect, and assistance by practical advice to producers concerned in their work of protectio n.

Section 3

Control of the Government Phytosanitary Service

The Government Phytosanitary Service is under the general control of the Royal Hungarian Ministry of Agriculture. [The actual department of the ministry that deals with this subject is the Phytosanitary Section IX. 4.]

Section 4

Departments of the Government Phytosanitary Service.

1. Consultative Body.
   Phytosanitary Council.
2. Experimental Body.
   Royal Hungarian Phytosanitary Institute.
3. Administrative Bodies.
   (*a*) Royal Hungarian Phytosanitary Service.
   (*b*) Royal Hungarian Phytosanitary Districts.
   (*c*) Local Phytosanitary Agents.

Section 5

The Phytosanitary Council is required to make recommendations to the Royal Hungarian Minister of Agriculture on any special question asked by this latter, and should make its proposals for its decisions.

The organization and Regulations of the Phytosanitary Council will be established by a special decree of the Royal Hungarian Minister of Agriculture.

Section 6

The Royal Hungarian Phytosanitary Institute, as the central body, has the following tasks :—

Theoretical and practical study of insects and parasitic plants, fungi and germs harmful to agricultural and garden produce, damage caused by biological change, as well as conditions of life of parasites and weeds.

Improvement on methods of protection, examination of preventative measures and protective machinery, laboratory examination and practical experiments, on different protective substances.

Organization on the ground of means of destruction of insects or parasitic plants which have made serious inroads in certain districts, by enlisting public participation or by asking for help from the State.

Practical advice and gratuitous special explanation to government authorities or private individuals concerned in the farm or garden produce.

Assistance in the collection of practical publications on plant protection organization of public lectures, systematic communication by broadcasting of actual phytosanitary questions.

Publication of an Annual containing the results of the Government Phytosanitary Service.

The organization and rules for the interior management of the Royal Hungarian Phytosanitary Institute will be laid down by a special decree from the Royal Hungarian Minister of Agriculture.

Section 7

The objects of the Royal Hungarian Phytosanitary Service, as the central administrative body, are as under :—

General supervision of phytosanitary affairs of the country, phytosanitary examinations in relation to the international import and export trade in agricultural produce (fruit, potatoes, onions, staddles, vine-shoots, seedlings, slips).

The conducting of examinations on the ground and the taking of measures in case of phytosanitary prohibition ; phytosanitary control and the inspection of establishments concerned with the cultivation, improvement, and trade in agricultural and garden produce (nurseries, seed depots, horticultural establishments, and other establishments for improvement of produce) ; the supervision of the enforcement of compulsory protective measures (extirpation) against insects and harmful plants ; Examination of Phytosanitary control as regards improved plants (seeds, bulbs) and with the verification and registration of the kinds permitted by the State ; compilation and preparation of statistical returns on damage caused by inclemency of the weather, insects and harmful plants, based on official phytosanitary reports ; instruction of district officials and directors, as well as personnel employed on phytosanitary experiments. Encouragement and direction of propaganda for the protection of plants against harmful insects and parasitic plants ; control of the special activity of District Phytosanitary Administrators and examining bodies, and the compilation of statistics on their actions, assistance in the publication of the Government Phytosanitary Service Annual and composition of practical manuals.

The central office of the Royal Hungarian Phytosanitary Service is Ministry of Agriculture V, Kossuth Lajoster 11, Budapest. Telegraphic address ; Plantval Budapest.

The organization and rules for the interior management of the Royal Hungarian Phytosanitary Service will be established by a special decree from the Minister of Agriculture.

Section 8

The Phytosanitary Districts, in their capacity as exterior administrative bodies, have the following tasks :—

Constant supervision of the phytosanitary condition of their area ; the conducting of phytosanitary experiments and qualifications ; reports to the Royal Hungarian Phytosanitary Service of the extent of damage caused by

insects, harmful plants, or the inclemency of the weather ; the commencement of practical and necessary work of a local character on phytosanitary affairs either on their own initiative or in accordance with orders received ; to stimulate with the above objects, local authorities in the enforcement of laws and decrees, and the putting into practice of the required measures ; advice to these authorities ; the submission of proposals to higher authority. Explanation and advice to agricultural producers, voluntarily and on demand ; intermittently, the arrangement of public lectures ; and finally the development of the greatest effort in the cause of phytosanitation within their sphere of activity.

Furthermore, it is their duty to direct and control any work of local phytosanitary agents, to develop their technical knowledge, and to keep themselves acquainted with their reliability and good will, and to prepare in the course of the above a general report summing up the detailed reports of these officials (Service of Information).

They are to correspond with regard to their reports, requests, and proposals direct with the Royal Hungarian Phytosanitary Service in cases of emergency or when circumstances are those of imminent danger ; information can be sent direct to the Royal Hungarian Minister of Agriculture by telegraph or telephone.

The organization of Royal Hungarian Phytosanitary districts is as below :—

Royal Hungarian phytosanitary districts are organized in administrative areas, in the centre of which, or close thereto, should be a winter school of agriculture, a special agricultural school, a secondary school of agriculture, a school of vine cultivation or cenology, or other public instructional establishment in agriculture or horticulture. Special districts are organized in towns with a municipality in big agricultural centres with large areas of cultivated ground from the agricultural and horticultural point of view.

District Directors are chosen from school or Agricultural Institute professors, selected by the Royal Hungarian Minister of Agriculture for instructing on the corresponding subject or from Phytosanitary Service, personnel employed in schools or other public agricultural establishments. Any areas not yet possessed of such a school or establishment will be provisionally attached to the nearest district.

Section 9

Local phytosanitary agents, as employees in the local Government administration, have the following objects :—

The encouragement of the special activities of district phytosanitary directors, and to place in hand work of local phytosanitary interest.

The organization of local phytosanitary agents is as below :—

Local phytosanitary agents are nominated yearly by the Royal Hungarian Minister of Agriculture from agriculturists, vine dressers and gardeners of suitable education. Their range of action should extend over the whole or a part of the area of a town, parish, or parish area, as may be suitable to Royal Hungarian phytosanitary districts.

Section 10

Information Service

To ensure the working of the Government Phytosanitary Service, its information service should be developed to a high degree of efficiency. The information service is both normal and special.

Normal communications include all about damage caused by inclemency of the weather, the importance and devastations of harmful insects and the best known parasitic plants. In accordance with the character of the damage or the agents that cause it, these communications should take place at intervals of varied length.

Special communications take place whenever certain pests, known or unknown, act unexpectedly on a large scale. Such communications should be incidental.

Section 11

The collaboration of Civil Authorities in phytosanitary matters.

The directing agents of the Government Phytosanitary Service should maintain close co-operation with the civil authorities involved, and should be at the disposal of the latter as regards their special opinion.

In return the civil authorities should guarantee uninterrupted work to members of the Government Phytosanitary Service.

Section 12

The task of the Government Phytosanitary Service in the realms of international affairs is as below :—

To ensure the fulfilling of their obligations as regards international trade in plants, and also obligations foreshadowed in the clause of Art. XVII of 1936 on the subject of the insertion of the International Agreement of Rome, dated April 16th, 1929, on plant protection, as well as those foreshadowed in other laws and decrees with regard to plant protection (section 2) and those required by foreign states in international trade of this nature.

Phytosanitary examinations laid down by international agreements in export and import traffic in farm produce are carried out by the Royal Hungarian Phytosanitary Service, and by special agents nominated by the Royal

Hungarian Minister of Agriculture. By the help of these specialists, export produce is graded, and marked with qualification signs. Any attestations related to this subject should be made in the name of " Royal Hungarian Phytosanitary Service."

Section 13

The present decree on the subject of international trade in plants will come into force on January 1st, 1937, and as regards internal trade in November 1st, 1936. Consequently, Decree No. 49,000/1932 F. M. in the rules of the Royal Hungarian Service for the protection of plants becomes null and void as regards international trade in plants, with effect from January 1st, 1937, and as regards internal trade with effect from November 1st, 1936.

Ferenc MARSCHALL, Dr.
m.p.
Secretary of State,
(for Minister).

BUDAPEST ;
October 27th, 1936.

LIST OF SPECIALIST OFFICIALS AUTHORIZED TO SIGN PHYTOSANITARY CERTIFICATES AS LAID DOWN BY THE GOVERNMENT PHYTOSANITARY SERVICE IN THE KINGDOM OF HUNGARY.

MARTON ACZEL	Official in special employment.
LASZLO AGH	Agriculturist with diploma. (Official in special employment.)
GUSZTAV AIGNER	Director of Royal Hungarian Special School of Agriculture.
JANOS AMBRUS	Professor of Agriculture. (Delegated director.)
ISTVAN BAGOTAI	Supernumerary Agricultural Official. (Delegated assistant.)
GABOR BAKO	Director General of Experimental Agricultural Research.
LASZLO BALAZSOVICH	Agriculturist with diploma. (Official in special employment.)
PAL BALPATAKY	Supernumerary Official in Vine Cultivation.
ISTVAN BARRA	Supernumerary Official in Experimental Agricultural Research.
FERENC BARANYOVITS	Supernumerary Official in Experimental Agricultural Research.
JOZSEF BENEDEK	Professor of Agriculture.
JOZSEF BONA	Sub-professor of Agriculture.
ENDRE BORY	Inspector of Agriculture (retired).
LASZLO CZAJLIK	Sub-professor of Royal Hungarian Agriculture.

Aladar Cziraky	Agriculturalist with diploma (in special employment).
Istvan Csabai	Supernumerary Agricultural official.
Bela Csanady	Professor of Agriculture (retired.)
Jeno Cseh	Sub-professor of Agriculture.
Lajos Cserey	Inspector of Horticulture.
Endre Csete	Professor of Agriculture.
Sandor Csete	Chief-assistant in Experimental Agricultural Research.
Gyorgy Csongradi	Professor of Agriculture (delegated director).
Zoltan Csorba	Supernumerary official in experimental agricultural research.
Beno Diera	Inspector General of Horticulture (director).
Istvan Dobo	Sub-professor of Agriculture.
Erno Dravetzky-Koos	Professor of Agriculture.
Tibor Erdelyi	Professor of Agriculture.
Andras Ferke	Horticulturalist with diploma (in special employment).
Laszlo Gajary	Professor of Agriculture (delegated director).
Istvan Galamb	Inspector General of Vine Cultivation.
Denes Gabos	Professor of Agriculture (delegated director).
Janos Gaman	Sub-professor of Agriculture.
Ifj. Istvan Gergely	Sub-inspector of Horticulture.
Janos Gergely	Horticulturalist with diploma (in special employment).
Zoltan Gero	Assistant in Experimental Agricultural Research.
Janos Giber	Professor of Agriculture (delegated director).
Janos Gollner	Assistant in the Royal Hungarian Academy of Agriculture.
Ferenc Gruber	Supernumerary Agricultural Official.
Ferenc Gucsy	Sub-professor of Agriculture (delegated director)
Antal Gulyas	General professor in the Royal Hungarian Academy of Agriculture.
Kornel Gyenes	Sub-inspector of Vine Cultivation.
Gyula Hajos	Inspector of Horticulture.
Istvan Hajos	Supernumerary Agricultural Official.
Gyula Halmos	Supernumerary Official of Vine Cultivation.
Istvan Harkai	Professor of Agriculture.
Erno Hemela	Sub-inspector of Vine Cultivation.
Kalman Hinfner	Supernumerary Official in Experimental Agricultural Research.
Vitez Ference Horvath	Chief Chemist in Experimental Agricultural Research.
Vitez Ivan Horvath	Professor of Agriculture (delegated director).
Sandor Imre	Professor of Agriculture (delegated director).

Andor I'so	Agriculturalist with diploma (supernumerary official).
Istvan Janko	Inspector of Vine Cultivation.
Viktor Kaczian	Agriculturalist with diploma (in special employment).
Gyula Kacsmarik	Professor of Agriculture.
Gyula Kadocsa	Director of Experimental Agricultural Research.
Geza Kapitany	Professor of Agriculture (delegated director).
Gyorgy Kelecsenyi	Sub-professor of Agriculture.
Hermann Kern	Director General of Experimental Agricultural Research, and member of upper council of rural economy.
Laszlo Ketter	Supernumerary Agricultural Official.
Gyorgy Komlossy	Assistant in Experimental Agricultural Research.
Viktor Kosinszky	Supernumerary official in experimental agricultural research.
Zoltan Kovacs	Director of Royal Hungarian Special School of Agriculture.
Jozsef Kranitz	Inspector General of Horticulture.
Jozsef Krenner	Chief Assistant in Experimental Agricultural Research.
Ferenc Kriszta	Sub-inspector of Horticulture.
Aladar Laczko	Chief Assistant in Experimental Agricultural Research.
Vitez Bela Lomjapataky	Inspector General of Vine Cultivation.
Bela Ludany	Sub-professor of Agriculture.
Gabor Maroti	Supernumerary Agricultural Official.
Denes Marton	Inspector General of Vine Cultivation.
Ifj. Peter Matusovits	Agriculturalist with diploma, in sepcial employment.
Janos Matrai	Professor of Agriculture.
Sandor Matray	Agriculturalist with diploma (administrator of Farm land cultivation).
Imre Meiszner	Inspector of Vine Cultivation.
Gyula Meszoly	Horticulturalist with diploma.
Jozsef Mikes	Supernumerary Agricultural Official.
Bela Mocsy	Supernumerary Agricultural Official.
Laszlo Molnar	Inspector of Agriculture (retired).
Aladar Neth	Professor of Agriculture.
Jeno Nemeth	Professor of Agriculture.
Zoltan Nemethy	Director of Royal Hungarian Special School of Agriculture.
Ivan Okalyi	Inspector of Horticulture.
Miklos Orincsay	Sub-professor of Agriculture.
Gyula Orosz	Professor of Agriculture (delegated director).

Lajos Palvolgyi	Supernumerary Agricultural Official.
Bela Pettenkoffer	Agriculturalist with diploma, in supernumerary employment.
Vitez Ferenc Peterdy	Professor of Agriculture (delegated director).
Laszlo Gyorgy Polgar	Inspector of Horticulture.
Janos Regele	Sub-professor of Agriculture.
Dezso Revy	Special professor of the Royal Hungarian Agricultural Academy.
Lajos Ripka	Inspector of Horticulture.
Laszlo Santha	Chief Assistant in Experimental Agricultural Research.
Gyorgy Sarospataki	Agriculturalist with diploma in special employment.
Jeno Sebestyen	Professor of Agriculture (delegated director).
Istvan Sebeok	Professor of Agriculture.
Sandor Seemann	Professor of Agriculture (delegated director).
Frigyes Seiffert	Specialist Official.
Jeno Suhajda	Sub-professor of Agriculture.
Miklos Szabo	Auxiliary Manager of Horticulture.
Jozsef Szajbely	Special Professor at the higher school of Horticulture.
Gusztav Szelenyi	Assistant in Experimental Agricultural Research.
Lajos Szentgyorgyvari	Director of Royal Hungarian Special School of Agriculture.
Lazar Szentirmay	Control Official.
Geza Szent-Galy-Faur	Supernumerary Agricultural Official.
Dezso Szilagyi	Inspector of Vine Cultivation.
Jeno Sziranyi	Inspector General of Vine Cultivation.
Janos Szirmai	Agriculturist with diploma (in special employment).
Endre Szlavik	Inspector of Horticulture.
Bela Szombath	Professor of Agriculture (delegated director).
Ifj. Kalman Tabajdi	Chief Assistant in Experimental Agricultural Research.
Gyorgy Terray	Supernumerary Vine Cultivation Official.
Agoston Toth	Supernumerary Agricultural Official.
Janos S. Toth	Sub-professor of Agriculture.
Istvan Urbanyetz	Supernumerary Official.
Jeno Urbanyi	Assistant in Experimental Agricultural Research.
Miklos Yadanyi	Sub-professor of Agriculture.
Jeno Vadnai	Professor of Agriculture (delegated director).
Sandor Vancsay	Professor of Agriculture.
Geza Varga	Professor of Agriculture (director).
Antal Varkonyi	Inspector of Vine Cultivation.

GYULA VEGH	Director of Royal Hungarian Special School of Agriculture.
LAJOS VEGHELYI . . .	Chief Assistant in Experimental Agricultural Research.
GYULA VENISS	Professor of Agriculture (delegated director.)
ISTVAN VIGH	Chief Assistant in Experimental Agricutural Research.
BELA WEITNER	Agriculturalist with dipoma, in special employment.
JENO GYORFFY	Chief Assistant in Experimental Agricultural Research (retired).
KORNEL KALLAY. . . .	Sub-professor of Agriculture.

REVIEWS

Soilless Growth of Plants. By Carleton Ellis and Miller W. Swaney. Reinhold Publishing Corporation, 330 West, 42nd Street, New York, N. Y. (1938). Pp. 155. Price $2·75 net.

The extremely interesting subject—soilless growth of plants, that is, the art of growing plants without soil—has so long been merely a matter of scientific interest and scattered information on this subject could be had only from scientific publications which ordinarily are neither very accessible nor intelligible to a layman. The growing of plants in culture solution is well known to students of plant physiology and only very recently there has been a move on the part of a few enterprising gardener-scientists to exploit the commercial possibility of the technique. In recent years brief articles of a popular nature on this subject have occasionally appeared in the leading papers of Great Britain and the United States; but to the most people these are yet nothing but a sensational piece of news of somewhat doubtful utility.

Messrs. Ellis and Swaney whose contributions to the development of the soilless technique are well known, have attempted for the first time to place before the general public the details of growing plants without soil. It is the first complete treatise which discusses thoroughly the three important methods of soilless growth—water culture, sand culture and sub-irrigation. In the present work they have expressed in popular language all the relevant information on the subject for the use of horticulturists and amateur gardeners. In eight chapters they have surveyed the whole process of soilless growth of plant, beginning from a general discussion on the chemistry of plant life to the formulae of nutrient solutions which are required for plant metabolism. The subject matter of the book is likely to appeal both to laymen and scientists, specially because the authors have not shown any tendency to indulge in high-sounding schemes, more sensational than practical, but have presented the subject as a scientifically sound and workable method.

Wherever there is too much pressure on the land and the climatic conditions are adverse to the production of green fodders and flowers all the year round, the technique of soilless plant growth will open up new possibilities. These methods will, therefore, undoubtedly prove attractive to American and Continental gardeners or farmers, specially in such localities where greenhouse raising of flower plants and vegetable is a commercial proposition. For obvious reasons, the ultimate value of the methods described in the book will be very limited, almost negligible, under present Indian conditions.

Although the underlying principles, as detailed by the authors, are equally of interest both to the layman and the scientist, a word of caution is necessary regarding the universal application of the methods. Different species of

flowers or vegetables have not the same nutrient requirements, and for efficient growth and development every species should have its specific nutrient. It is, therefore, somewhat hazardous to treat all types of vegetation by standard methods. Unfortunately, nowhere in this book have the authors clearly drawn attention to this fact, nor to the fact that considerable amount of work yet remains to be done before the methods described could be widely applied. In their attempt to present facts in non-technical language, the authors have naturally steered clear of discussions of a technical nature, which detracts much from the real value of the book to scientific readers.

Altogether the book is likely to catch popular imagination and the authors have successfully unmasked the 'mystery' of growing plants without soil.

There are in the book certain loose expressions like 'rest period in plant', which seems to have been applied with a view to appeal to the imagination of non-technical readers. In such publications scientific accuracy or discourses of highly technical nature are not always possible, as that would, as apprehended by the authors, 'tend only to confuse the reader or cause him to lose interest.'

The printing and the general get-up of the book are excellent. (B. K. M.)

Plant Injection for Diagnostic and Curative Purposes (*Technical Communication 10 of the Imperial Bureau of Horticulture and Plantation Crops, East Malling, Kent, England*). By W. A. ROACH. Foreword by PROF. V. H. BLACKMAN: 1938, pp. 78, plates 2, text-figures 47, bibl. 162. Price 5*s*.

MAN has toyed for centuries with the idea of feeding his fruit trees direct instead of by more orthodox but slower methods and in the heyday of the Italian Renaissance we find Leonardo da Vinci noting that if it is desired to produce poisoned fruit the injection of fruit trees offers a way.

More recently various 'tree doctors' have suggested that easy rejuvenation of orchards can be effected by the use of their own particular injection methods, but such methods have always been somewhat suspect, since obviously different conditions demand different remedies and the cure may prove worse than the disease.

It has remained for Dr. Roach to bring order out of chaos and show those of us who are attracted by the idea how to set about our investigations.

He starts by giving a history of injection work in Europe and the U. S. A., paying particular attention to that published in recent years on the detection and cure of deficiency diseases. He notes also the successful use of injection for purely physiological purposes, as, for instance, of glucose into vines, just before bud break, to ensure a good fruit set. He points out, moreover, how

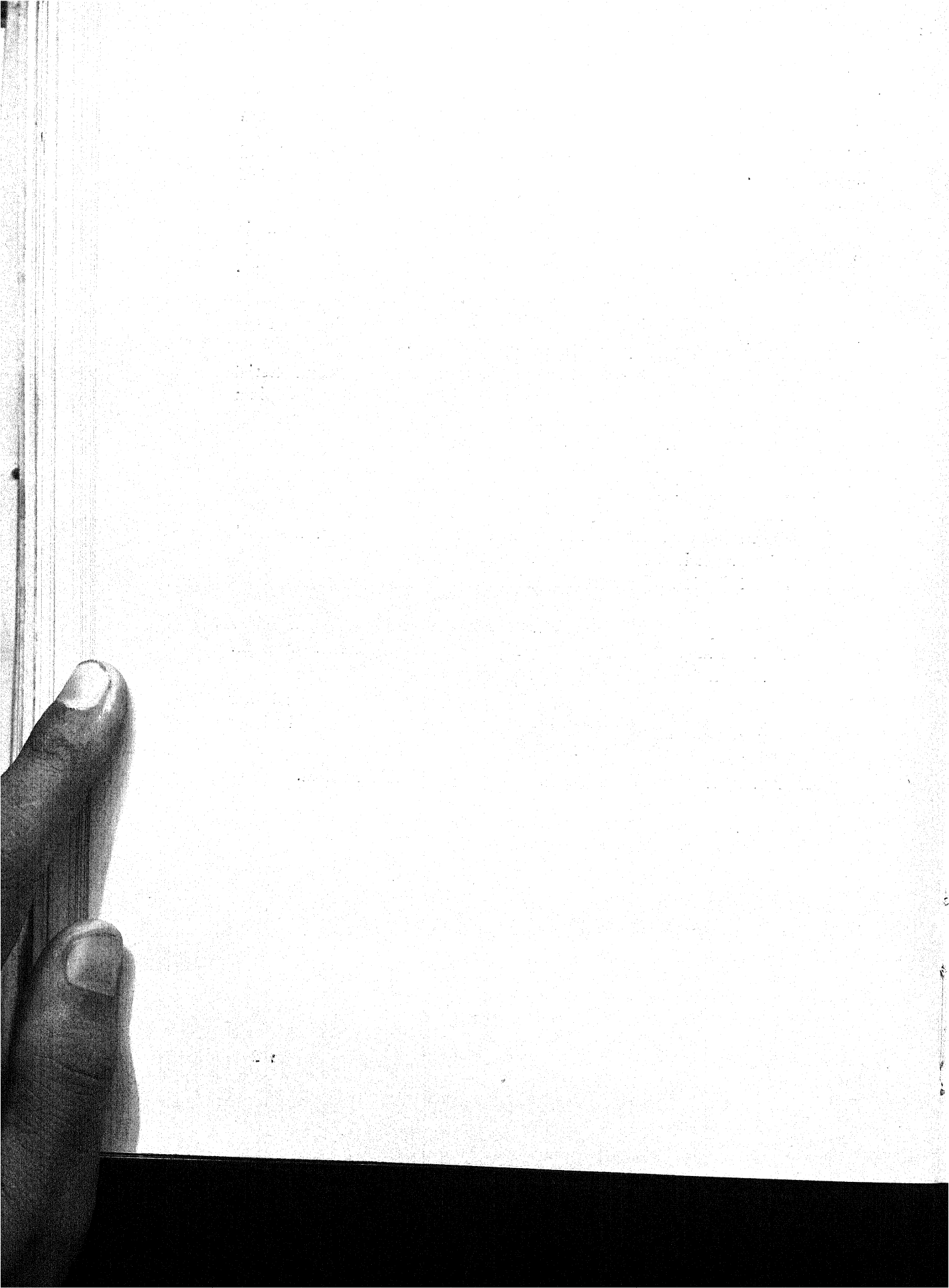
injection methods may be of great value in the study, not only of mineral deficiencies, but also of rootstock influence and of the effect of the chemical constituents of a fruit on its storage capacity.

Recent cases of successful injection cited include the following diagnosis of incipient chlorisis of the iron shortage type at East Malling; diagnosis and cure of a copper deficiency dieback disease in apples in Western Australia; determination of the cause and cure of boron deficiency diseases of apples in Canada and New Zealand; increase in vigour and freedom from insect pests in apple trees injected with various solutions in England.

His own experiments have been in progress for seven years on material which has ranged from the strawberry plant to fully grown apple and plum trees, and most of the present paper is devoted to a consideration of the methods used. It is for this clear and illustrated account that horticulturist will be particularly grateful. He describes in detail with the help of text-figures and lists of tools the technique used for the injection of particular parts of standing trees and other plants, varying in size from a single interveinal area of a leaf to whole main branches, noting, moreover, the type of problem for which each particular method is best suited. He shows how in the most delicate methods the injection of leaves and their comparison with neighbouring un treated leaves or parts of leaves enables a rapid diagnosis to be made of the mineral deficiency from which a plant or tree is suffering. He describes how whole trees may be injected for experimental purposes or even for economic reasons in the commercial orchard.

In short he shows us how to carry out the operations advocated and how to avoid those many pitfalls which await the over-zealous, although he is far from suggesting that all his methods are perfect.

He invites criticism and the experience of others in the same field.



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ORIGINAL ARTICLES

THE GENUS *FUSARIUM*

II. A SPECIES OF *FUSARIUM* AS A CAUSE OF WILT OF GRAM (*CICER ARIETINUM* L.)

BY

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(Received for publication on 23rd December 1938)

(With Plate XVIII)

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I. Introduction

Wilt of gram (*Cicer arietinum*) is a common disease in many parts of India where this important crop is grown on a wide scale. It has been reported as being particularly serious in Madras [Narasimhan, 1929] and has been noted in Bengal [Hedayetullah, 1937]. Although the disease has been known for a long time it has not been extensively studied. Wilted plants were received at Pusa (Bihar) from Burma in 1920, a year in which the crop had been severely damaged. Isolations were made from the wilted plants and species of *Fusarium* were obtained in culture. Attempts to reproduce the disease with these isolates failed [Mitra, 1925] but inoculations with a later isolate were partially successful [McRae, 1932]. It was thought by McKerral [1923] that the disease was soil-borne and could be avoided by long rotations with other crops.

Narasimhan [1929] proved that gram wilt could be caused by a species of *Fusarium*. He described the fungus but did not name it. It is not possible from the description given to name the species with confidence.

In 1935 Dastur reported that wilt had caused serious damage to the gram crops at Nagpur, Central Provinces, and he found that *Fusarium* was associated with almost every wilted plant, but he was unable to prove the pathogenicity of the fungus and considered it to be a saprophyte living in the soil. He also obtained *Rhizoctonia bataticola* (Taub.) Butl. in culture and found that it was responsible for slight damage. However, the true wilt, he concluded, was due to unfavourable soil conditions. By repeatedly sowing the crop in the same place he found that it was affected year after year. While suggesting that wilt was of physiological origin he was not clear as to the exact nature of the ailment.

During the past two seasons (1936-37, 1937-38) the gram plots at the Imperial Agricultural Research Institute at Delhi have suffered death of plants before reaching maturity up to the extent of ninety per cent, and most of the plots have shown at least twenty-five per cent loss. Observations made at the Agricultural Sub-Station at Karnal showed that of the eighty-four types grown almost all showed severe damage. The material for this study was collected at these two centres in 1937 and 1938.

II. Symptoms

The disease was generally observed in the field when the crop was about one month old, and the number of dying plants was usually greatest when they were two or three months old. The plants could be divided into three groups according to the symptoms they produced :—

(1) Those in which the first symptom was drooping of the upper leaves followed soon by the lower leaves. The plants withered and died within about a week.

(2) Those in which the leaves gradually turned yellow and then began to drop, the remaining leaves rapidly withering and the plant dying.

(3) Those in which the leaves became red. In the later stages these plants resembled those of group (2).

The disease was not limited to isolated plants but was found in patches of various sizes in which a high percentage of plants was killed.

When sections were made of the roots and stems of the three different forms of wilted plants all appeared alike. Fungal hyphae were numerous in the vascular tissues of all plants showing typical symptoms of wilting. The number of hyphae in a vessel varied greatly, some vessels containing only a single strand while others were completely filled. In some plants in the last stages of infection the cortex of the roots was heavily invaded by mycelium. The cell-walls of the phloem tissue of infected plants appeared to be considerably thickened and the colour of this tissue was often dark brown.

Hyphae could be seen high up in the stems but were never observed in the petioles of leaves.

III. Isolation

Two methods of isolation were used :—

(*a*) Portions of roots and stems, half to one centimeter in length, were thoroughly washed in water and were immersed in 0·1 per cent mercuric chloride solution for five to ten minutes. They were then transferred aseptically to sterilized water and were washed three or four times in separate lots of water. Finally they were transferred to slopes of oatmeal agar.

(*b*) Similar portions of roots and stems after preliminary washing in water were transferred to a one per cent aqueous solution of silver nitrate. After about two minutes they were removed and immersed in a sterile one per cent solution of sodium chloride to precipitate the disinfectant, according to the method described by Davies [1935], and were transferred as soon as possible to agar slants.

From almost all pieces of infected tissue *Fusarium* grew out in the course of about a week or ten days. There were few other fungi. The isolates of *Fusarium* varied markedly in many characteristics. Since over three hundred isolates of *Fusarium* were obtained many of which appeared alike, it was found necessary to reduce greatly the number for detailed examination. It was found that this could be done rapidly (without the use of a microscope) by grouping them into types, according to the major characteristics, notably the kind of spore-bearing structures and alteration of the colour of the medium. Thirteen such groups were formed, and many members of the various groups were then rapidly examined microscopically to determine whether there were marked differences within the groups. Only slight differences were observed, indicating that the selection based on microscopic characters was fairly reliable for the purpose of reducing the number of isolates for further study. The thirteen types obtained are described briefly in Table I.

Table I

Types of fungi isolated from gram

Type No.	Aerial mycelium	Discoloration of medium	Spore-bearing structures
1	Whitish, abundant	Brown	Sporodochia
2	White, fluffy	None	None
3	Pink	Pink	None
4	Purple	Purple	None
5	Pink	Carmine	None
6	Dark purple	Dark purple	None
7	White	None	Thin layer on surface of agar
8	White, scarce	None	Ditto
9	White	Pink	None
10	White	Pink	Green sporodochia
11	Buff	Pink	None
12	White	Brown	Pale yellow sporodochia
13	Pink	Pink	Slimy mass

In Table II are listed the numbers of isolates of the various types obtained at each date of isolation, together with the source of the material.

TABLE II

Numbers of isolates of each type obtained

Type No.	Number of cultures of isolates made on 4-3-37 (Karnal)	Number of cultures of isolates made on 27-11-37 (Delhi)	Number of cultures of isolates made on 9-12-37 (Karnal)	Number of cultures of isolates made on 18-1-38 (Delhi)	Total number of isolates (Both centres)
1		10		6	16
2		43	5	19	67
3		15		5	20
4		13	28		36
5		10		7	17
6		9			9
7		26		12	38
8		16			16
9	37		10		47
10	15		15		30
11	18				18
12	9			3	12
13	4				4

An effort was made to isolate *Fusarium* from the seeds of wilted plants. After surface-sterilizing with silver nitrate solution and washing with sodium chloride solution, they were plated on potato dextrose agar. No fungous growth was found on these dishes after one month, indicating that the fungus was not internally seed-borne.

IV. INFECTION TESTS

Experiment 1

Inoculum was prepared of types 1—8 by growing them in flasks of 100 grams of paddy moistened with 150 c.c. of water and sterilized by autoclaving at twenty pounds pressure for one hour. Four flasks were prepared of each type. After three weeks, when good growth had taken place (on January 25th, 1938) nine-inch pots were two-thirds filled with sterilized soil,

and on the surface of each was sprinkled half the contents of one flask. Ten gram seeds were placed on this inoculum, the remainder of the inoculum was scattered on the seeds, and the pots were covered with $1\frac{1}{2}$ inches of sterilized soil. Controls were prepared by sowing seeds between layers of sterilized soil and paddy. The pots were removed to a bird-proof shelter and kept watered.

The seedlings emerged through the soil ten days after sowing in the case of the controls and those sown in soil containing inoculum of types 1, 2, 7 and 8, but in the other cases no germination occurred. Thirty-two days after sowing, wilting was observed in some plants inoculated with type 2. First the uppermost leaves drooped, then the wilting spread to the lower leaves. Eventually nearly half of the plants in this series of pots died. Eight days after wilting first appeared with type 2, the plants started to wilt with types 7 and 8, and eventually some plants in these pots also succumbed. The experiment was terminated on March 7th by which time the plants were becoming too large for the pots which contained them. The final figures obtained are given in Table III.

TABLE III

Germination and wilting with isolates of types 1 to 8

Type of isolate	Total number of seedlings	Number of seedlings which eventually wilted	Germination percentage	Wilting percentage
1	39	0	97·5	0
2	30	13	75	43·3
3	0	..	0	..
4	0	..	0	..
5	0	..	0	..
6	0	..	0	..
7	38	6	95	15·8
8	38	5	95	13·2
Control	38	0	95	0

The seeds were dug up from the pots in which no germination had taken place and it was found that they were completely rotten. It is clear from this experiment that the *Fusarium* species studied fall into three groups, non-pathogenic (type 1), wilt-producing (types 2, 7 and 8) and seed rotting (types 3, 4, 5 and 6). Sections made from stems of wilted plants revealed abundant

hyphae in xylem vessels and cortex. Re-isolations made from stems and roots of infected plants showed in all cases only fungi resembling those with which they were inoculated.

Experiment 2

This experiment was performed in a manner similar to the previous one except that instead of using paddy the inoculum was prepared from a sterilized mixture of 190 grams of soil, 10 grams of maize-meal and 60 c.c. of water. The fungi, including all types from 1 to 13 were grown for one month. Eight pots of each type containing six seeds were prepared, 200 grams (dry weight) of inoculum being added to each pot. Four of the pots were daily watered with 200 c.c. of water while the other four received only 100 c.c. The seeds were sown on March 1st, 1938.

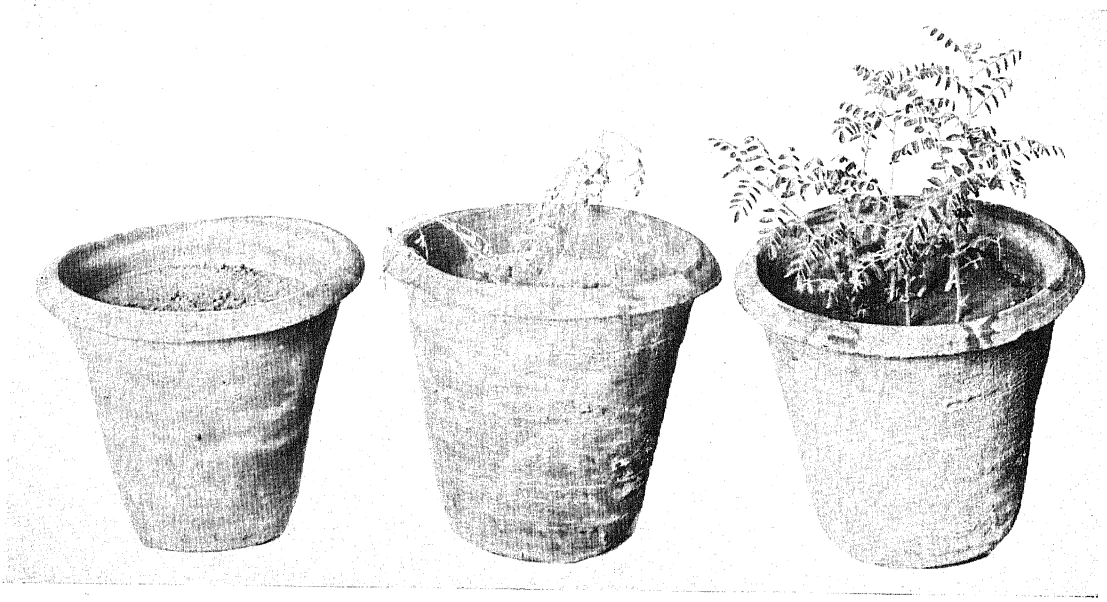
Seedlings emerged well in the controls and with types 1, 2, 7, 8 and 12 in about six days ; but in the remaining types germination was very poor, most of the seeds on being dug up were found completely rotten.

Seventeen days after sowing, the pots inoculated with type 2 began to wilt in the low water series, and two days later in the high-water series. Wilting first appeared with types 7 and 8 shortly after type 2. Types 1 and 12 caused no wilting and the plants appeared as healthy as in the controls. The germination and the final figures for wilting with the various isolates in the dry series taken on completion of the experiment on March 25th are given in Table IV. The figures for the wet series are omitted as they were essentially the same as the dry series. Plate XVIII shows pots from the seed-rotting, wilting and control series.

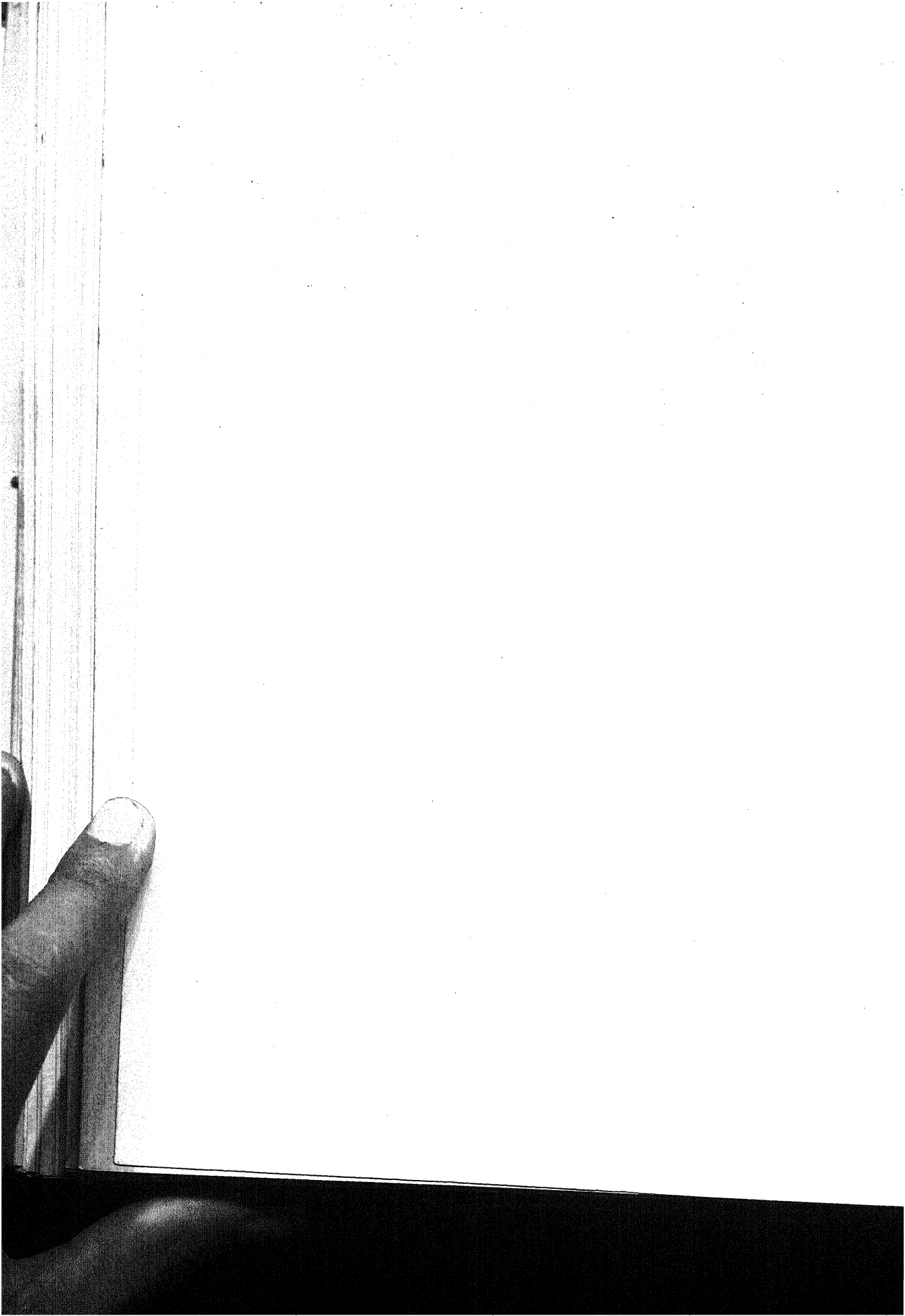
TABLE IV

Germination and wilting with isolates of types 1 to 13 after 25 days

Type of isolate	Total number of seedlings	Number of seedlings which wilted	Germination percentage	Wilting percentage
1	24	0	100·0	0
2	23	23	95·8	100·0
3	4	0	16·7	0
4	2	0	8·3	0
5	1	0	4·2	0
6	0	..	0	..
7	24	9	100·0	37·5
8	22	7	91·7	31·8
9	0	..	0	..
10	4	0	16·7	0
11	0	..	0	..
12	24	0	100·0	0
13	0	..	0	..
Control	24	0	100·0	0



Pots of gram inoculated with seed-rotting (left) and wilt-producing (centre) *Fusaria*. Control pot at right



The results confirm entirely those of the first experiment, and indicate that types 9 to 13 showed no wilting, but all these except number 12 caused severe rotting of seeds, preventing germination.

Experiment 3

In view of the fact that heavy inoculations with certain types of *Fusarium* caused seed-rot it was thought that these when applied in lesser quantities might also produce a wilt. Inoculum of type 4 was therefore prepa red in the usual manner from cornmeal-soil mixture. It was applied to pots of sterilized soil in layers above and below the seed as before, the amounts of inoculum in various series being 200, 100, 50 and 25 grams. After three weeks no germination had taken place in any of the inoculated plots, and the seeds were found rotten in the soil, while the controls had produced healthy seedlings. As far as the dilutions went, therefore, they did not result in causing wilt. Further reductions in quantity would be necessary in order to say for certain that the symptoms of wilt cannot be reproduced by these fungi.

V. Morphology of the wilt fungus

Isolates of types 2, 7 and 8, all of which caused wilting, were selected for further study. Cultures of each were made by selecting germinating single spores from dilution plates of plain agar.

The three isolates were grown at a temperature of about 25°C. in tubes of the following media prepared according to the methods of Wollenweber *et al.* [1925] :—

Oat agar.

Potato dextrose agar with two per cent dextrose

Potato dextrose agar with five per cent dextrose

Potato cylinders.

Steamed rice.

Colours of the mycelium were noted and named according to Ridgway [1912] after fourteen days' growth. Measurements of 200 spores were taken after twenty-one days' growth. The colours are recorded in Table V, and lengths of conidia in Table VI. No sporodochia were formed, nor were there any signs of sclerotia. A plectenchymatous layer somewhat resembling a stroma was formed on the surface of the agar tubes, but it is questionable whether this could be called a genuine stroma since it was not a raised, cushion-like spore-bearing structure. There appeared to be a concentration of spores on the surface of the agar media and potato cylinder in the case of type 8 and on two per cent potato dextrose agar with type 7, but it would perhaps be stretching the meaning of the term to refer to this concentration as 'pionnotal'. Terminal and intercallary chlamydospores were produced by type 2 on oatmeal agar and steamed rice, and by type 7 on all five media, but none were produced by type 8.

TABLE V

Colours of types 2, 7 and 8 after 14 days of growth

Kind of medium	Type No. 2		Type No. 7		Type No. 8	
	Aerial mycelium	Plecten-chymatous layer	Aerial mycelium	Plecten-chymatous layer	Aerial mycelium	Plecten-chymatous layer
Oatmeal agar .	White	White	White	White	Lacking	White
Two per cent potato dextrose agar.	,,	,,	,,	,,	,,	,,
Five per cent potato dextrose agar.	,,	,,	,,	,,	,,	,,
Potato cylinder	,,	Ochraceous buff	,,	Orange buff	,,	,,
Steamed rice .	,,	,,	,,	,,	,,	,,

TABLE VI

Measurements of 200 spores of the three isolates after 21 days of growth

Type No.	Septation group	Number of spores in group	Range (μ)	Mean (μ)
2	0	200	7—15×2—5	9·9×3·2
	1	0		
	2	0		
	3	0		
7	0	128	6—15×2—5	10·2×3·1
	1	50	14—21×2—4	17·5×2·4
	2	22	19—28×2—5	23·4×3·2
	3	0		
8	0	86	7—15×2—5	11·3×3·2
	1	61	15—21×2—5	18·6×3·4
	2	21	19—30×2—5	26·8×3·2
	3	32	28—64×2—6·5	43·2×3·6

VI. Discussion

The isolations carried out in this incomplete study of the gram wilt disease have been insufficient to establish with any certainty what types of *Fusarium* are generally associated with the disease and the results of the study on standard *Fusarium* media have not been given in sufficient detail to enable a final conclusion to be drawn as to the species of *Fusarium* which caused wilt, and certain rather surprising results have been withheld pending confirmation. Some very important points have emerged, however, and this is a good opportunity to take stock of the situation.

One thing is definitely established. Whether or not a wilt disease of gram may be caused by some physiologic disturbance due to unfavourable soil conditions, as believed by Dastur [1935], it is now proved that it may be caused by *Fusarium* and the work of Narasimhan [1929] is confirmed.

Secondly, it is clear that wilted gram plants may harbour an extraordinarily wide variety of *Fusarium* types, perhaps different species, and these are capable of causing two distinct diseases, a seed-rot and a wilt. This is reminiscent of certain diseases of other legumes, notably peas (*Pisum sativum*) and pigeon-peas (*Cajanus cajans*). The complexity of these diseases has been referred to in recent times by Padwick [1938] and Wollenweber [1938]. In such cases it is necessary to make a careful survey of material from many sources before starting to generalise either about the cause of the disease or about the subsequent control measures. The fact that there are fungi causing a pre-emergence blight and that this is quite distinct from the wilt disease in which there is no reduction at all in stand invites attention to the remark of McKerral [1923] to the effect that in Burma severe cases of disease in gram, when it is grown year after year in the same place, result in failure of the seedlings to emerge through the soil. He also may have been dealing with a complex disease.

Thirdly, it is certain that all the three wilt-producing isolates briefly described, although different in minor respects, notably in septation and length of conidia, nevertheless are alike in major characteristics. The abundance of small conidia—' Kleine Konidien ' of Wollenweber and Reinking [1935] and not true ' microconidia ' of Brown [1928] which are non-septate and not pear-shaped, the presence of intercallary and terminal chlamydospores, and the delicate cell walls, place at least isolates numbered 2 and 7 in the section Elegans, while the absence of sporodochia and of pionnotes (at least of unmistakable pionnotes) and the ratio of length to breadth (12·0 :1) of spores where such exist brings us to the conclusion that all three isolates are of the sub-section Orthoceras.

From this point it is useless to speculate further. The basis for the identification of *Fusarium* species of the section Elegans is too much in dispute to attempt to name the three isolates we are studying. We have given above only the barest details of our examination of the isolates and a mass of data is now being accumulated in which other species of the sub-section are being compared with these isolates under varying conditions. According to Nelson,

Coons and Cochran [1937] we would be in our rights in calling the three isolates a new species and it would certainly be easier to name them thus rather than attempt to distinguish them on grounds of morphological differences of doubtful validity. What is required is a fair understanding of the range of variability of the isolates in regard to (*a*) environment and (*b*) strain differences. In the present state of knowledge, in fact, it only appears possible to identify these fungi by comparison under identical conditions with all known species of the sub-section. It is a formidable task but there is no alternative and this work is being undertaken.

VII. Summary

(1) Isolations were made from wilted plants of gram (*Cicer arietinum*) at Delhi and Karnal (Punjab).

(2) Over three hundred isolates of *Fusarium* were obtained and on rough inspection appeared to fall into thirteen types.

(3) Representatives of the thirteen types were tested for pathogenicity by infesting sterilized soil. Eight types caused severe seed-rotting, three caused wilting and two appeared to be non-pathogenic.

(4) Examination of single-spore cultures of the three types which caused wilting revealed that at least two were closely related forms belonging to the sub-section Orthoceras. Their exact relationship to one another is being studied.

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SUGARCANE IN THE PUNJAB, II

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(With two text-figures)

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PREFACE

THE authors wish to record their appreciation and acknowledgment of the valuable assistance given by the Deputy Directors of Agriculture and their staff, of the Lyallpur, Montgomery, and Multan circles in carrying out these surveys. They also wish to acknowledge the ready co-operation offered by Major G. V. A. Prideaux, Officer Incharge Military Farms, Okara, and other zamindars in the areas covered by this survey in reserving plots of cane for analysis and in giving other valuable help.

The surveys described in this paper were carried out by Dr. Girdhari Lal and Ch. Fateh Mohammad in the Lyallpur and Sheikhupura districts and L. Jiwan Das Chopra and S. Asghar Ali in the Lahore, Montgomery and Multan districts. Special credit is due to S. Asghar Ali and Ch. Fateh Mohammad for help in the arrangement and computation of the data relating to the large number of analyses carried out.

P. E. LANDER,
RAMJI NARAIN

LYALLPUR ;
9th December, 1937

Introduction

In 1934 the authors of this paper published Part I of "Sugarcane in the Punjab" [Lander *et al.*, 1935], which gave an account of a survey of the sugarcane in the Gurdaspur, Sialkot (sub-mountainous tracts), Jullundur and Hoshiarpur (central Punjab), and the Karnal and Rohtak districts of the

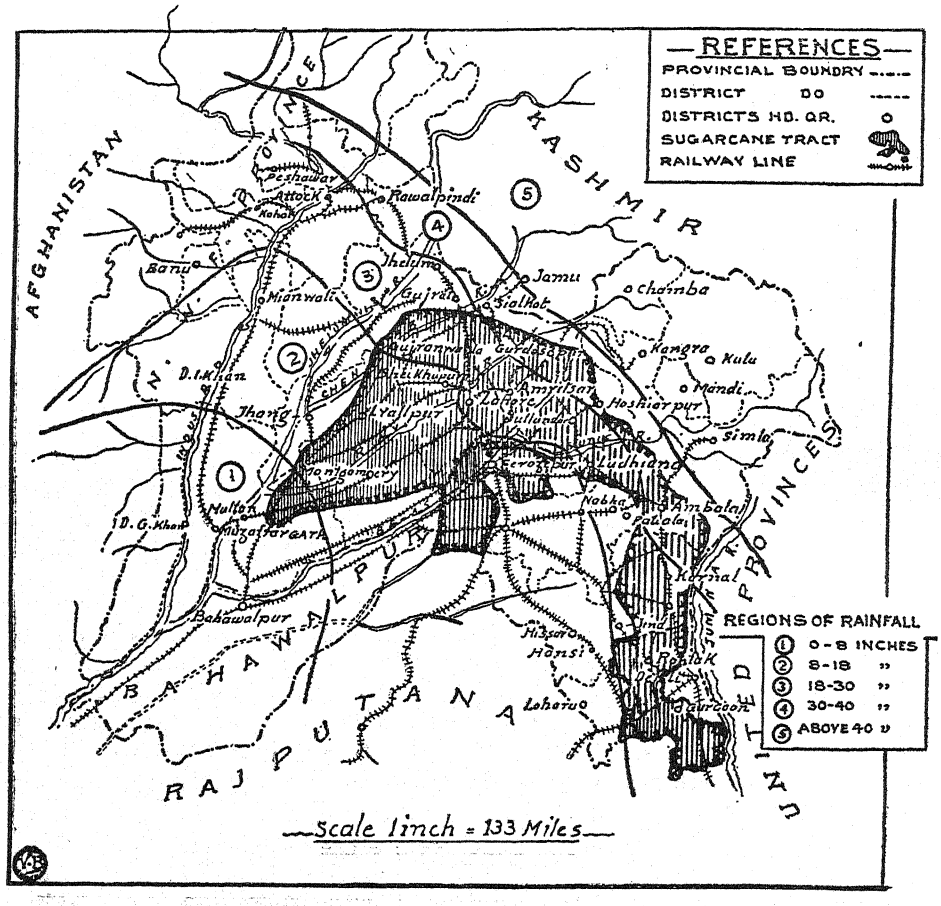

Fig. 1. Map of the Punjab showing main sugarcane tract including districts with an acreage of 10,000 or above, and distribution of average rainfall

south-eastern Punjab. The object of this survey originally was to ascertain those local varieties of sugarcane and the new Coimbatore varieties which would be most suitable for supplying the then increasing number of sugar factories with canes maturing over a period which would ensure an economic working period for the factories. Attention was directed particularly to such aspects as the periods of ripening of the various canes, with tonnage or yield per acre both in stripped cane and juice, the quality of the juice, the effect

of frosts and insect pests and diseases on the juice, and the variations of these and a number of other factors which depended on, or might be influenced by, the geographical situation of the areas surveyed and the prevailing agricultural practices in those areas. The data which was derived from the first survey published in Part I appeared to be sufficiently valuable to justify extending this survey to the Lyallpur, Sheikhupura, Lahore, Montgomery and Multan districts in which very considerable areas are under sugarcane. The present publication gives an account of this survey which was carried on during the cane seasons from 1933-34 to 1936-37, on lines similar to those described in Part I.

Lyallpur District

General physical and climatic conditions

The Lyallpur district lying between latitudes 30°-48′ and 31°-48′, and longitudes 72°-10′ and 73°-48′ constitutes the most important agricultural tract in the new Canal Colonies and includes about 40,000 acres of cultivated land under sugarcane and is thus only second in importance and extent of acreage to Gurdaspur. The annual rainfall is low, being only about 12 to 13 inches per annum, most of which falls in July and August. The soil, fuller particulars of which have been given by the authors elsewhere [Lander, Ramji Narain and Mukand Lal, 1929], consists mostly of medium loam and is inclined to be light and sandy rather than heavy clay and yields high outturns, both in cane and other crops suited to its latitude, with the application of organic manures and also some artificials combined with adequate irrigation. Although so much sugarcane is produced in this district and others of the Punjab, it would be inaccurate to state that the Punjab is particularly suitable for this crop, as in certain districts, of which Lyallpur is one, frost is a regular phenomenon in December and January at a time when its effect on the cane crop is likely to be most adversely felt. The occurrence of frost exercises an unfavourable effect on the general condition of the crop, which effect may be minimised or increased by certain other conditions relating to the current agricultural practices of which the time and extent of irrigation is one.

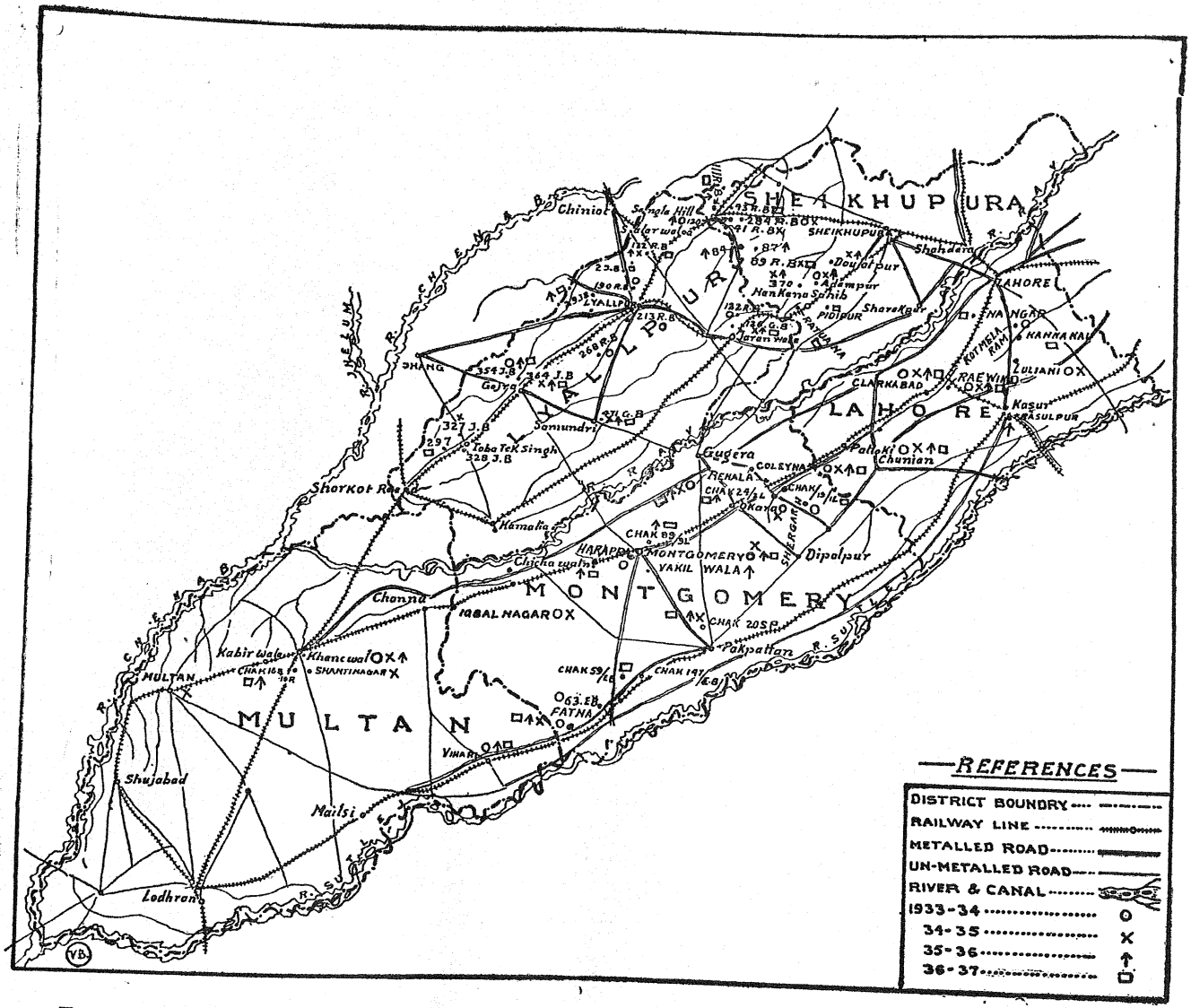

FIG. 2. Map of districts Lyallpur, Sheikhupura, Lahore, Montgomery and Multan showing villages under observation (1933-36)

Rotations of crops

The rotations most commonly to be found in this district are :—

1. Maize—Sugarcane—Wheat.
2. Maize (*Senji*)—Sugarcane—Wheat.
3. Cotton—Sugarcane—Wheat.
4. Wheat—Fallow—Sugarcane—Cotton.

Farmyard manure is the manure usually applied to sugarcane and the only one which can safely be said to yield an economic return considering the cost of the manure applied. Owing to the very light rainfall in this district the number of irrigations usually given to the sugarcane crop is high, being from fifteen to twenty during the season.

Varieties of sugarcane

Katha, a local variety, is still extensively grown and as late as last season (1936-37) it occupied 86 per cent of the total area under cane in this district.

Katha is a thin cane and gives a low yield averaging about 12·8 tons of stripped cane, 7·42 tons of juice and 1·64 tons of total solids per acre. These figures represent roughly yields of about 2/3rd those obtained from the new Coimbatore varieties. Katha is cultivated in this district more for fodder than for sugar.

Ponda, one of the oldest varieties in the province, is not grown to any considerable extent in the Punjab colonies as, being a thick and juicy cane, it requires a comparatively larger quantity of water for its cultivation and more care than the newer Coimbatore varieties. The most common Coimbatore varieties grown in this district are Co 223 and Co 285, but other Coimbatore varieties which are being introduced are Co 290, Co 312, Co 313 and Co 331. Co 205 was the earliest of the newer varieties to be introduced, but this is now being replaced by others which are superior both in the quality of the juice produced and the tonnage yield per acre, and which have the additional advantage of ripening earlier than Co 205. Another variety, *viz.*, Co 223 is also losing favour with the cultivators as this is a soft cane which has proved to be particularly susceptible to attack by wild animals, insects and various diseases peculiar to cane, and it is also unfavourably affected by frost. The canes which appear to be most suited to the general climatic and other conditions of the Lyallpur district are Co 285, Co 312 and Co 313.

Coimbatore varieties, in addition to being heavy yielding canes, are also suited to the practice of ratooning and on this account are finding increasing favour amongst zamindars.

Period of optimum ripeness

The period of optimum ripeness starting with Katha, the local cane, extends from the 3rd week of November, when harvesting usually begins, to the first week of April, although at certain places the incidence of rain in Decem ber and January may assist in prolonging the crushing season until the las-

week of April. Co 213 is usually the last of the Coimbatore varieties to ripen and becomes fit for crushing about the end of December and remains so until April. Varieties which ripen somewhat earlier than this are Co 223 and Co 285. The introduction of the Coimbatore varieties has considerably extended the period during which the canes of the district are ripe and in suitable condition for factory operations, but this period cannot usually be extended beyond the end of March because by this time the cultivator wishes to prepare his fields for sowing cane and cotton for the coming season and to harvest and thrash his wheat. In order, therefore, to cater for these factors it is necessary to introduce early-ripening canes in the general farming practice in addition to those which are still unripe during the period when frost occurs, *viz.*, from the end of December to the beginning of February, as well as to select varieties which withstand the effects of frost. Speaking generally, analyses of different varieties of cane at present grown in the Lyallpur district show that they are mature and are fit for crushing from the end of November to the middle of April (Table IV).

Two recent Coimbatore varieties which have done well in certain tracts of the Province and also at the Government Farms at Lyallpur and Risalewala are Co 312 and Co 313, but these varieties have not yet been taken up to any extent on zamindari lands. Their more extensive use is however, adovcated as they are high yielders and the analytical data indicate that they are resistent to frost.

Quality of cane

The quality of the juice produced from any particular variety of cane is a most important factor from the point of view of the sugar factory owner. A high quality juice should show high purity values and a low glucose ratio, combined with a low mineral content. Data on the first two of these values are shown in Tables I—III.

Qualitative and quantitative comparison of indigenous and Coimbatore varieties

The relative value of these two types of cane is summarised in averages obtained from the data of the survey in the statement below

Variety	Stripped cane	Juice	Total solids	Sucrose	Glucose ratio	Purity coefficients
			Outturn in tons per acre			
Local . .	12·8	7·42	1·55	1·27	1·7	82
Coimbatore .	18·32	11·76	2·14	1·75	2·8	82
			Outturn per ton of cane			
Local . .	..	0·58	0·121	0·099	..	..
Coimbatore .	..	0·64	0·117	0·096	..	..

This statement shows that Coimbatore varieties are superior in most aspects to the local Katha. The slight difference in the glucose ratio in favour of the local Katha is more than compensated for by the high yield of stripped cane and sucrose from the Coimbatore varieties.

Effect of frost

During the four years of this survey no severe attacks of frost were experienced and therefore it is difficult to say how these new varieties would have withstood frost in the districts surveyed. There was a slight incidence of frost in the 1934-35 season but no extensive ill effects were noted, and in almost all cases recovery was complete. Lander and Ramji Narain [1935] have shown that in the central and southern districts of the Punjab, of the three varieties Co 205, Co 213 and Co 223, Co 213 showed almost complete immunity to frost whilst Co 205 and Co 223 were only slightly affected. It might be expected therefore, that these varieties will respond to frost in a similar way in the districts now being described.

SHEIKHUPURA DISTRICT

This district, which lies between latitudes 31°-11′ and 32°-4′, and longitudes 73°-16′ and 74°-46′, comprises 2,312 square miles, and adjoins Lyallpur on its south-western side and Lahore on its south-eastern side, was created in 1919 from the districts of Lyallpur, Gujranwala and Lahore. In the last year of the survey, *viz.*, the season 1936-37, this district had about 20,000 acres under sugarcane.

General physical and climatic conditions

The climatic features of the Sheikhupura district more closely resemble those of Lahore and Gujranwala than those of Lyallpur. It receives an annual rainfall of from 15 to 18 inches. Its soil is mostly a medium loam, but owing to the rise of the sub-soil water there is a tendency, at a number of places, for the soil to develop saline conditions or become even alkaline. The normal soil of the district, however, yields a cane crop which for corresponding varieties appears to be distinctly superior to that of the Lyallpur district, a feature which may in some measure be due to the higher rainfall. The general agricultural practice followed is much the same as in the Lyallpur district, and the manurial practices and rotations followed are also similar.

Varieties of cane

Hitherto Katha and Ponda have been the two chief varieties grown, but in recent years the area under improved Coimbatore varieties has been steadily increasing from 15 per cent of the total area under cane in 1932-33 to 32 per cent in 1936-37. This increase of improved varieties in the Sheikhu pura district is very pronounced in comparison with the corresponding in crease in the Lyallpur district which in the last year of our survey (1936-37 had only 13 per cent of its total cane area under improved varieties. In the Sheikhupura district Katha has given an average of 16 tons of stripped cane per acre and Ponda 28·9 tons. These figures are higher than the corresponding

figures for the Lyallpur district which were 12·8 and 26·2 respectively. The most common Coimbatore varieties grown in the Sheikhupura district during the survey were Co 205, Co 213, Co 223, and Co 285, but during the last two years Co 244 and Co 313 were also introduced. As in the Lyallpur district, Co 205 and Co 223 appear to be losing favour with zamindars, whilst Co 285 is being more extensively developed.

Period of optimum ripeness

The period of optimum ripeness for the local Katha and Coimbatore varieties extends from the 3rd week of November until the middle of April. Of the Coimbatore varieties, Co 313 ripens about two weeks before the local Katha, and during the season of 1936-37 it yielded 70 per cent more stripped cane per acre than Katha. Co 313 remained in a condition fit for crushing until the middle of April, and owing to these favourable qualities it appears to be the most likely variety to replace Katha, hitherto the main cane grown by zamindars in the new Colony areas. Of the other Coimbatore varieties investigated in this district, Co 223 and Co 244 were ripe about the 3rd week of November, whereas Co 213 ripened in the middle of December. It appears therefore that with a judicious combination of the new Coimbatore varieties the cane crushing season can conveniently extend from the beginning of November until the midle of April. The convenience of the zamindar, however, does not as a rule permit him to keep his cane standing in the fields so late in the season (Table VIII).

Quality of the cane

Cons dering the factors which are involved in the term ' quality ' , Ponda appears to be the best cane of all those examined in this district. Of the Coimbatore varieties, Co 223 and Co 313 (1936-37 only) gave the highest purity figures, but Co 213, Co 244 and Co 285 were almost as good. The main features pertaining to the composition of all these canes are shown in Tables V and VI, whilst the average mean composition and the yields per acre of all varieties examined in the district during the entire period of the survey are given in Table VII.

Comparison of indigenous and Coimbatore varieties

The relative value of these two varieties are summarised in the statement given below :—

Variety	Stripped cane	Juice	Total solids	Sucrose	Glucose ratio	Purity coefficient
			Outturn in tons per acre			
Local . . .	16·0	9·02	1·83	1·50	1·3	82·0
Coimbatore .	22·9	14·84	2·69	2·25	3·0	83·7
			Outturn per tons of cane			
Local . . .	..	0·564	0·114	0·094	..	..
Coimbatore .	..	0·647	0·117	0·098	..	..

From the point of view of sugar and *gur* manufacture (Ponda is mostly used for chewing), the quality of juice of Katha indicates that it is one of the best canes grown, but the above statement shows that the local Coimbatore varieties are equally good and have the additional advantage of yielding about 50 per cent more stripped cane, 75 per cent more juice and 45 per cent more sucrose per acre than Katha.

Lahore District

General and physical conditions

The Lahore district lies between latitudes 30°-38′ and 31°-44′, and longitudes 73°-38′ and 74°-58′, and grows only 5 per cent of the total sugarcane produced in the Punjab. It consequently cannot be regarded as so important a cane-growing tract as are some others. Its importance, however, lies in the fact that it grows Ponda to an extent not found elsewhere. Its climate varies somewhat from that found in the Lyallpur and Montgomery districts, due to its higher rainfall of from 18 to 21 inches per annum, which has the effect of producing somewhat higher average humidity. The Lahore district shows a natural division between the central uplands which include portions of the three tehsils of Chunian, Lahore, and Kasur and the alluvial lands which lie between the Ravi, Sutlej and Beas rivers. These two tracts are respectively designated by the terms 'Sweet Majha' and 'Saline Majha' (Majha Mitha and Majha Khara). In the latter of these as the name implies, the soils and well waters have a tendency to salinity. The rainfall also is a very uncertain factor. These adverse climatic conditions and soil features are mitigated, however, to a considerable extent by canal irrigation. Consequently the agricultural conditions of the district are not disturbed to any great extent. Cane samples concerning which data are given in this paper were taken from both these tracts, although most of them came from the Majha Khara.

Crop rotations

The rotations usually followed are the same as those of the adjoining district of Sheikhupura, except where some modifications are introduced on well-irrigated areas. Heavy applications of farmyard manures are usually applied to Ponda cane in the well-irrigated areas and to a somewhat lesser extent in the canal areas. Maize is the crop in the general rotation which is always heavily manured, although manure is also applied to some extent before sowing sugarcane. The number of irrigations given to the cane crop usually depends here, as elsewhere, on whether it lies in the canal or well-irrigated tracts, and also to some extent on the amount of rainfall. In this district lands which are subject to river action are seldom if ever manured.

Varieties of cane

As already mentioned Ponda is the chief variety grown in this district and is mainly used for chewing, although some *shakkar* is also made from it. Katha and Kahu are the only thin indigenous varieties cultivated. Ponda gave the

highest tonnage of stripped cane and sucrose per acre of all the varieties investigated, the average figures for four years being 28·8 tons and 3·19 tons respectively or about 60 per cent more than was obtained from the Coimbatore varieties. Of the latter, Co 285 appears to be the best, whilst Co 312 which was only introduced in the 1936-37 season gave very poor yields. Other Coimbatore varieties met with were Co 205, Co 223 and Co 290.

Period of optimum ripeness

The harvesting of cane generally begins in the Lahore district about the middle of November, although most of the canes investigated during the survey were not ripe until the end of November. The shortest period of optimum ripeness was noticed in the case of the thick varieties, Ponda and Red Stripped Mauritius, and extended from the beginning of December to the end of March. The longest period extending to more than four months was found with Co 285 and Co 312, but as already remarked Coimbatore canes in this district are not as extensively grown as is Ponda, although there seems to be no reason why they should not do well under careful cultivation. The data in Table XII show that canes in this district are not generally ripe until early December, a fact which may probably be due to the somewhat more moist conditions found here.

Quality of cane

The figures for purity coefficient and glucose ratio of Co 312, although available only for one year, indicate that this variety is one of the most promising in the district, while the same factors show that Co 205 is least satisfactory. The rest of the Coimbatore varieties appear to be of equal merit in this respect (Tables IX and X).

Yields of cane

In computing the yields from the Lahore district during the survey it has been found that some of the Coimbatore varieties exhibit a distinct susceptibility to any curtailment in the water supply. For example it was found, for reasons which we need not enter into here in detail, that canes on the District Board Farm at Raewind suffered from a curtailment in the water supply available in the seasons 1934-35, 1935-36, and 1936-37, with subsequent low yields. Not all varieties suffered equally in this respect, Co 312 and Co 290 being most affected. The above-mentioned fact is illustrated by the figures given in Table XI. If we exclude from our calculations those varieties which suffered from the curtailment of the water supply, then the average figures for yields of the Coimbatore varieties in the Lahore district compared favourably with corresponding figures obtained from the other districts surveyed.

The data for these two groups are shown in the two statements below :

Comparison of indigenous and Coimbatore varieties (excluding Ponda which is not used for sugar making)

*Statement (a)**

Variety	Stripped cane	Juice	Total solids	Sucrose	Glucose ratio	Purity coefficient
			Outturn in tons per acre			
Local . .	8·7	5·14	1·00	0·84	2·9	83·7
Coimbatore .	15·2	9·73	1·76	1·51	3·4	85·9
			Outturn per ton of cane			
Local . .	..	0·59	0·115	0·097	..	..
Coimbatore .	..	0·64	0·116	0·099	..	..

* When the drought-affected canes are included

Statement (b)†

Variety	Stripped cane	Juice	Total solids	Sucrose	Glucose ratio	Purity coefficient
			Outturn in tons per acre			
Local . .	8·7	5·14	1·00	0·84	2·9	83·7
Coimbatore canes excluding Co 290 & Co 312	18·7	11·7	2·09	1·76	4·3	84·4
			Outturn per ton of cane			
Local . .	..	0·59	0·115	0·097	..	..
Coimbatore .	..	0·63	0·112	0·094	..	..

† When the drought-affected canes are omitted

It will be seen that the outturn for the Coimbatore canes is slightly less than double that of the local varieties. It has already been observed that the zamindars of the Lahore district devote most of their energies to the production of Ponda which from their point of view is a more economic proposition as a chewing cane for the neighbouring towns than cane for sugar production.

Montgomery District

General and physical conditions

This district lies approximately between latitudes 29°-58′ and 31°-33′, and longitudes 72°-30′ and 74°-58′, and cannot really be considered a sugarcane growing district of the Province, as it is only within recent years that the cultivation of cane has been taken up to any appreciable extent.

Prior to 1920 the average area under cane in this district was only about 700 acres. From that time there has been a steady increase in the area under cane except during the cotton boom years, 1926-29, when the price of cotton was very high. Since 1930 the area under cane again increased, and in the 1931-32 season, the figure approximated 20,000 acres, after which there was a gradual decline, until, during the period covered by this survey it remained fairly steady between 12 and 15 thousand acres. There are two main divisions in the Montgomery district known as the Ganji Bar and the Nili Bar, respectively. The Ganji Bar which occupies the higher regions of the district was the first to be colonised about the year 1913, whilst the Nili Bar is still being colonised at the present time. Most of the survey work in this district was confined to the Ganji Bar.

The climate of the district taken as a whole is very dry and the mean annual rainfall about ten inches. Temperatures of 120°F. in the shade are common in the summer months, whilst as a contrast the winters are cold, the temperature frequently falling to freezing point and below. The soil of the Montgomery district tends to become lighter as we proceed from the north-east to the south-west, until near the boundary of the Multan district it is extremely light and sandy. The soil between Khanewal and Montgomery is on the whole a very good loam which produces excellent crops, but this area also contains some of the worst and most intractable alkaline lands in the Province, particularly in the Montgomery tehsil of the Ganji Bar tract. A conspicuous feature of this bad soil is a high percentage of sodium carbonate which has converted the normal calcium clay of the soil into a sodium clay.

Although the total amount of clay in the soil is not conspicuously high, yet the soil shows all the features of an excessively hard and imprevious clay.

The soils found in the Nili Bar are more uniform in mechanical composition than those in the Ganji Bar and vary from medium loam to light loam as one proceeds from east to west.

Rotations of crops

No definite and fixed rotations are followed by the zamíndars in this district, but generally speaking those which include sugarcane are much the same as are found in the Lyallpur colony. Farmyard manure is almost invariably applied to the cane crop in amounts varying from 400 to 500 maunds per acre. The number of irrigations given is approximately the same *viz.*, between 18 and 20 or perhaps slightly more than in the Lyallpur district.

Varieties of cane

Katha is the most extensively grown local variety, chiefly for fodder purposes, although Ponda is found to some extent mainly in the Depalpur tehsil. The area under local varieties is approximately two-thirds of the total area under cane in the district; the rest is under new Coimbatore varieties and is increasing in extent from year to year. The Coimbatore varieties most usually grown by the zamindars are Co 223, Co 285, and to lesser extent Co 213 and Co 290, whilst Co 205 is being gradually replaced by better varieties. The more recent improved varieties which are at present mostly confined to Government Farms and some large estates are Co 300, Co 301, Co 312 and Co 313. The last named appears to be one of the most suitable and promising canes and might conveniently replace Katha.

Period of optimum ripeness

Katha (period of ripeness 11th November to 10th March) and Co 285 (period of ripeness 28th November to 9th April) are the two canes which ripen earliest of those generally grown by the zamindar. Thus with a combination of these two varieties it should be possible to extend the crushing season from early November to approximately the middle of April. The season might be extended to the middle of April or even longer if Co 300, Co 301 and Co 313, which are generally ripe from the middle of November until the middle of April, and Co 210, Co 213 and Co 270, which are ripe early in December, could be included.

Quality of cane

It will be seen from Tables XIII to XV that the order of yield for stripped cane among the Coimbatore varieties is Co 312, Co 301 and Co 285, and their quality is approximately equal, although Co 301 appears to yield a slightly higher percentage of sucrose on cane. Judging from the percentage of sucrose on cane, Co 313 is the best cane although it yielded only 19·3 tons of stripped cane per acre. Its glucose ratio was also the lowest of all the canes examined. It is interesting to record that, taking averages, the Coimbatore varieties Co 301, Co 312 and Co 313 gave roughly 0·5 tons more sucrose per acre than the older Coimbatore varieties. The figures for Co 285 and Co 223 were 2·08 and 1·80 tons of sucrose per acre respectively.

Comparison of indigenous and Coimbatore varieties

The statement below shows the relative figures in outturn per acre for the local and Coimbatore varieties :—

Variety	Stripped canes	Juice	Total solids	Sucrose	Glucose ratio	Purity coefficient
			Outturn in tons per acre			
Local . .	13·0	7·54	1·60	1·30	1·8	81·5
Coimbatore .	19·1	12·42	2·27	1·93	2·3	85·6
			Outturn per ton of cane			
Local . .	..	0·43	0·123	0·100	..	..
Coimbatore .	..	0·65	0·119	0·101	..	..

MULTAN DISTRICT

General and physical conditions

This district lies between latitudes 29°-22′ and 30°-45′, and longitudes 71°-4′ and 72°-55′. It has an excessively hot dry summer extending from April to September with a rainfall of six inches or less per annum, and can hardly be regarded as a suitable tract for the extensive cultivation of cane, which is mainly found in the Khanewal tehsil bordering the Montgomery district. Ponda is grown to some extent near the towns in the Multan tehsil for chewing purposes. The rotations and general agricultural practice followed are much the same as those of the adjoining Montgomery district. Farmyard manure is applied to sugarcane, which in the Khanewal tehsil is almost entirely irrigated by canal water, whilst such cane as is grown in Multan and Shujahabad tehsil is irrigated by well water, the level of which lies about 25 to 30 feet from the surface.

Varieties of cane

After Ponda (the chewing cane), Katha is the most important local variety found, whilst Co 205, Co 223 and Co 285 are the only new varieties found on zamindars' lands. The data recorded in this paper on other varieties were obtained from the Agricultural Farm at Multan, the B. C. G. A. Farm at Khanewal, and the Government Seed Farm at Vihari. It is interesting to record that inspite of the adverse climatic conditions of this district the Coimbatore varieties have given, where irrigation and manuring facilities are adequately available, as high yields as in the Montgomery and Lahore districts

Period of optimum ripeness

During the four years of the survey, Katha was observed to be the earliest cane to ripen, about the middle of November, and remained in a condition fit for crushing until the 2nd week of April. Co 223 and Co 285 were ripe early in December, and Co 205 at the end of December. The other canes tested at the Government Farm at Vihari, *viz.*, Co 290 and Co 312, were ripe before the end of November, and Co 281 and Co 213 early in December. Agricultural requirements, however, rarely permit the cane to remain in the field later than the end of March. (Table XX).

Quality of the cane

The data presented in Tables XVII to XIX, give the figures for yield and composition for the different varieties studied, Co 285 grown under zamindari conditions appears to yield the highest tonnage of stripped cane and sucrose per acre, but its average purity coefficient and glucose ratio were low compared with the other Coimbatore varieties. Of these Co 312 gave the highest yields of stripped cane and sucrose per acre and it may be remarked that this variety did much better at the Agricultural Farm at Vihari in the seasons 1935-36 and 1936-37 than in the previous year at the B. C. G. A. Farm at Khanewal. Generally speaking, almost all the varieties did better at the Vihari Farm than in the Khanewal tehsil.

Comparison of indigenous and Coimbatore varieties

Variety	Stripped cane	Juice	Total solids	Sucrose	Glucose ratio	Purity coefficient
			Outturn in tons per acre			
Local .	14·4	8·18	1·71	1·45	1·1	85·2
Coimbatore .	19·1	12·20	2·19	1·87	2·3	85·3
			Outturn per ton of cane			
Local . .	..	0·57	0·119	0·101	..	..
Coimbatore .	..	0·64	0·115	0·098	..	..

On the whole, the Coimbatore varieties in this district have done better than the local ones, although not so well as in other districts. Katha however appears to have done better in the Multan district than in Montgomery or Lahore.

SUMMARY

1. The survey described in this paper was conducted for four years in the Lyallpur, Sheikhupura, Lahore and Montgomery districts and the Khanewal tehsil of the Multan district.

2. On account of intensive heat in summer and cold winters, more particularly the latter, the Lyallpur and Montgomery districts cannot be regarded as pre-eminently suitable for sugarcane, yet owing to canal irrigation the Lyallpur district which has more than 40,000 acres under cane ranks next to Gurdaspur, the premier cane-growing district of the Province. The data obtained during these surveys indicate that with adequate irrigation there appears to be no reason why cane growing could not be developed in the Montgomery district to the same extent.

3. In the Multan district, which is hotter and more arid than Lyallpur and Montgomery, the absence of irrigation facilities is a more serious limiting factor for cane growing than the high summer temperatures.

4. The hardier varieties which are better able to withstand extremes of temperature and scarcity of water are more suitable than the soft ones for the tracts described in this report.

5. Coimbatore varieties have invariably been found to give better yields of cane and sucrose per acre than the local varieties. The best yields from both local and Coimbatore varieties were obtained in the Sheikhupura district, whilst the yield from local canes in the Multan district (Khanewal) was the next best after Sheikhupura, and slightly better than Montgomery. Both at Montgomery and Multan, Coimbatore canes did equally well as regards yield.

It is interesting to record that the Lahore district, which appears to be one of the most suitable of all the tracts surveyed, gave the lowest yields of indigenous cane of any district in the Punjab, possibly due to the fact that zamindars there pay more attention to thick canes such as Red Striped Mauritius and Ponda varieties which are grown for chewing and fetch better prices. These thick varieties which are extensively grown in the Lyallpur district have been found to give better yields than the Coimbatore varieties. It was found that the greatest amount of sucrose produced from an acre of local cane was obtained in the Multan district, Sheikhupura, Lyallpur and Montgomery coming next in order. The Coimbatore varieties gave the highest yie'ds of sucrose per acre in Sheikhupura and Montgomery. The order in which the various districts have benefited most in respect of increased yields of sucrose per acre due to the introduction of new Coimbatore varieties appears to be Lahore, Sheikhupura, Montgomery, Lyallpur and Multan.

6. The Coimbatore canes, which have been introduced in the Punjab from time to time, have been steadily improving in quality as judged from the figures for sucrose percentage on cane and therefore the cultivators are now more eager to take to Coimbatore varieties than they were before.

7. Owing to very hot summers followed by the dry autumn months, sugarcane in the Lyallpur and Montgomery districts ripens comparatively earlier than in other districts of the Punjab excepting Sheikhupura, where for certain unexplained reasons, the crop is the earliest to ripen.

8. With the introduction of new varieties, the period of optimum ripeness has been considerably increased. In the Sheikhupura district where the canes

mature earlier than elsewhere the crushing season was found to extend over a period of 154 days, while in the Lyallpur, Montgomery, Multan and Lahore, it was 145, 146, 146 and 124 days respectively. The crushing season is considerably longer in these districts than in the central and south-eastern districts, due partly to the introduction of early ripening varieties of which Co 313 is the most important.

9. The districts covered by the present survey were found to be comparatively free from those diseases and pests which usually cause damage to the cane crop in the central and south-eastern districts. The only disease met with was red-rot, which was severe in the Chunian tehsil of the Lahore District and Depalpur of the Montgomery district. Top borer was found to cause serious trouble at the Montgomery, Fatna and Vihari farms. Both red-rot and top borer are at present localised and all possible efforts should be made to prevent their extension.

General observations on sugarcane in the Punjab

It might be of interest now to consider the figures for average outturn of stripped cane per acre and the amount of sucrose contained therein for the different cane-growing districts of the Punjab which have been considered in this paper and in Part I.

Table XXI below shows that the Rohtak and Karnal districts in the south-east of the Punjab have hitherto given the highest yields of stripped cane per acre both for local and Coimbatore varieties. The Jullundur and Hoshiarpur districts come next in order of yield. Of the remaining districts dealt with in these surveys the Lahore, Gurdaspur, and Sialkot districts have given the lowest yields of local canes, while the Lyallpur, Montgomery, Multan and Sheikhupura districts occupied an intermediate position in the order given.

The yields of the Coimbatore canes from the Sheikhupura district were approximately the same as for the corresponding varieties from Jullundur, whilst the remaining districts yielded about 19 tons of stripped cane per acre in each case. These figures indicate that the introduction of new Coimbatore varieties has been very profitable for those districts which have hitherto produced only low yields of local cane. The figures for the yields of sucrose per acre follow approximately the same order as those for stripped cane. It may be noted, however, that although less sucrose was obtained from the local canes in the Jullundur and Hoshiarpur districts than from those at Rohtak and Karnal, yet the two former districts gave the highest yields of sucrose per acre from the new Coimbatore varieties of any district in the Province. In other words, both the quantitative yields and the quality of the local varieties in the Rohtak and Karnal districts were superior to the local varieties in other tracts of the Province, and the Coimbatore varieties in the Gurdaspur and Lahore districts were superior to the local varieties both in tonnage yield and in quality.

It will be seen from Table XXIII that Gurdaspur had the largest area under sugarcane, Lyallpur coming next, Sialkot being second however in 1936-37. As already recorded, however, the Montgomery district has not hitherto been regarded as a suitable cane growing tract owing to high summer temperatures and some other unsuitable features. Although Lyallpur experiences approximately the same climatic conditions as Montgomery, yet the yields from both local and Coimbatore canes were better in Montgomery than in Lyallpur. There would appear, therefore, to be no valid arguments against the further extension of cane cultivation in the Montgomery district provided irrigation facilities and manure are available to the same extent as in Lyallpur.

An important matter which deserves close attention but upon which no extensive investigations have yet been carried out, concerns the influence of the nature of and the form in which the alkali bases occur in the soil, on the quality of cane in the Lyallpur, Montgomery, and other districts of the North-West dry area of the Province. The amount of exchangeable calcium present in the soil seems to exercise a great influence on the amount of mineral matter present in the juice. Data available at present indicate that owing to the low concentration of exchangeable calcium in the soil of these districts the juice of the canes gives an abnormally high mineral content which results in a decrease of approximately 10 per cent in the possible recovery of white sugar, compared with a corresponding recovery from the juice of the canes grown in the central tracts of the Punjab. Investigations on this problem have been in progress for some time and the results thereof will be published in due course, when it is hoped that some knowledge may be obtained of methods which may help to alleviate this defect in the canes in question.

Table XXII gives the periods of ripeness and length of the crushing seasons. Corresponding figures for the districts previously surveyed have also been included in this table. It is interesting to note that in every year of this survey almost all varieties of cane in the Sheikhupura district for some obscure reason, ripened earlier than elsewhere. Katha, generally ripens in the Punjab about the middle of November although in the Lahore district it is usually somewhat later. Ponda is usually ripe by the beginning of December. Of the four chief Coimbatore varieties grown in the Punjab *viz.*, Co 205, Co 213, Co 223 and Co 285, Co 205 usually ripen in early December in the western districts of the Punjab but not until early January in the central and eastern districts or even as late as the first week of February in Jullundur. Both Co 213 and Co 223 are ready by early December in the western districts, and by mid-January in the central and south-eastern parts of the Province. Correspondingly Co 285 is usually ready for crushing by the end of November in the western district and mid-January in the central. Considering the cane crop as a whole in the various districts of the Punjab, Sheikhupura, as already noted, stands first as regards early maturity of its cane which is usually ready by the 3rd week of November. Lyallpur and Montgomery follow roughly a week later, whilst Multan and Lahore districts show a still further delay of about one week. In the sub-mountainous regions represented by Gurdaspur

and Sialkot, and the central districts such as Jullundur, Hoshiarpur and the south-eastern districts around Rohtak and Karnal, Coimbatore canes as a whole are rarely ready for crushing before early January.

Length of crushing season

The length of the crushing season for factory or other requirements is obviously governed in the various districts by the periods over which the cane is in a ripe condition. An interesting fact brought out by the surveys recorded in Part I was that, although cane grown under zamindari conditions in the south-eastern districts of the Province gave higher tonnage yields than elsewhere, none of the varieties examined gave a crushing period of more than 100 days. The surveys of the districts recorded in this paper, however, show that in these there is a much wider choice of varieties extending over a longer crushing seasons so that it should be possible to depend entirely upon Coimbatore varieties to supply cane to the factory from the 3rd week of November until the middle of April, whereas in the sub-mountainous districts of Gurdaspur and Sialkot, the central districts of Jullundur and Hoshiarpur and the south-eastern districts of Rohtak and Karnal, it is necessary to depend upon low yielding indigenous early ripening varieties to permit a factory to operate till the Coimbatore varieties become available.

At the Agricultural Farm, Montgomery, the newly introduced Co 300, Co 301 and Co 313 permit a crushing season of more than 140 days, whilst in the Sheikhupura district Co 313 remained in a condition fit for crushing for 154 days. Considering the quality, tonnage, and length of crushing season provided by Co 313 this variety appears to be the most promising one for the districts considered in the present survey, and it is desirable to develop its cultivation more extensively in some of the tracts reported on in Part I to see if its favourable features would be maintained there also. Other varieties which appear to be most suitable for the tracts now reported on are Co 285 and Co 312.

Sugarcane as fodder

It is remarkable that Katha still occupies a very large portion of the total area under cane in the province particularly in the Lyallpur and Sheikhupura districts. The common belief of the zamindars that, in case of scarcity of fodder, this cane can be used as a substitute seems to be mainly responsible for its retention as it yields only about half the tonnage obtained from improved Coimbatore varieties otherwise it might have disappeared long ago. Another reason which zamindars sometimes advance is that Katha ripens much earlier and therefore the *gur* prepared from it in the early season can be sold at a premium. With the introduction of new fodders and new varieties of sugarcane both these reasons have lost their force. For winter berseem is a very good fodder crop and if systematic efforts are made to popularise this crop there is no reason why zamindars should grow Katha rather than the Coimbatore canes. In Co 313 which ripens as early as Katha and yields about three times as much, we have a cane which is an excellent substitute for Katha. It is essential therefore that systematic propaganda in favour

of new Coimbatore varieties should be carried out especially in the Lyallpur district which unfortunately has only about 13 per cent of its sugarcane area under new varieties.

Diseases and pests

The districts surveyed in this report were found to be comparatively free from those diseases and insect pests which usually cause such havoc in the central and south-eastern districts of the Province. The only disease which was found to any extent was red-rot which caused considerable damage to Co 213 and to a lesser extent to Co 223 in the 1933-34 season. In that year it was found to some extent in the Lyallpur and Sheikhupura districts, but was particularly severe in the Chunian Tehsil of the Lahore district where the crop was almost entirely destroyed in some of the fields preserved. In Chunian and in the Depalpur tehsils of Montgomery, red-rot has been steadily increasing in recent years and unless special efforts are made to control it by sowing sound healthy canes, and in cases where the disease has been very severe by burning the diseased crop, it is possible that the trouble may spread further.

Top borer caused serious though localised trouble in the Lahore and Montgomery districts where Co 223 and Co 312 were badly attacked at the Montgomery, Fatna and Vihari farms. As this pest is at present fairly localised, all possible efforts should be made to prevent its extension.

Ratooning

Ratooning is not practised in the districts under report to anything like the extent found in the sub-mountainous and central districts of the Province, but only as a rule in those localities where water is available at a nominal or no cost, such as the riverain tracts found in the Gurdaspur and Hoshiarpur districts. It was very seldom that ratoon crops more than one year old were found in the districts under report, although a feature of such crops is that they usually ripen from a fortnight to a month earlier than the planted crop of the same variety. As remarked in Part I, if the practice of ratooning is adopted, adequate measures of insect control must be carried out.

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TABLE I

Average composition of different varieties of sugarcane during period of maturity (Lyallpur District)

Year	Locality	Weight of stripped cane in tons per acre	Per cent on cane of				Glucose ratio	Purity coefficient
			Juice	Total solids	Sucrose	Glucose		
			Co 205					
1934-35	Chak 132 R. B.	1·35	61·8	10·80	8·20	0·65	7·9	76
1935-36	Chak 471 G. B.	16·00	57·0	10·80	8·83	0·25	2·8	82
1936-37	Chak 328 J. B.	17·20	61·5	11·15	9·13	0·37	4·1	82
Average for 3 years	..	15·85	60·1	10·92	8·72	0·42	4·8	80
			Co 213					
1933-34	Chak 213 R. B.	15·6	66·6	11·50	9·40	0·24	2·7	82
			Co 223					
1933-34	Chak 190 R. B.	18·82	63·9	11·60	9·60	0·40	4·2	83
1934-35	Chak 132 R. B.	16·70	66·3	11·63	9·58	0·31	3·2	82
1935-36	Chak 364 J. B.	15·10	63·7	12·00	10·13	0·28	2·8	84
1936-37	Chak 2 J. B.	23·60	66·4	11·91	10·00	0·30	3·0	84
Average for 4 years	..	18·55	65·1	11·71	9·82	0·32	3·3	83

TABLE I—*contd.*

Year	Locality	Weight of stripped cane in tons per acre	Per cent on cane of				Glucose ratio	Purity coeffi. cient
			Juice	Total solids	Sucrose	Glucose		
			Co 285					
1933-34	Chak 213 R. B.	31·2	62·0	12·30	9·80	0·17	1·7	80
1934-35	Chak 132 R. B.	17·5	64·1	12·18	9·60	0·18	1·8	81
1935-36	Chak 471 G. B.	16·3	60·5	11·70	9·60	0·18	1·9	82
1936-37	Chak 471 G. B.	21·7	62·3	11·80	9·81	0·20	2·0	83
Average for 4 years	..	21·7	62·2	12·00	9·70	0·18	1·9	81
			Co 290					
1934-35	Chak 327 J. B.	19·9	66·6	12·32	10·20	0·20	2·0	83
			Ponda					
1935-36	Chak 126 G. B.	26·2	69·8	13·5	11·68	0·35	3·0	87
			Katha					
1933-34	Chak 190 R. B.	13·7	54·9	11·20	9·30	0·06	0·7	83
1934-35	Chak 132 R. B.	10·8	60·6	12·50	9·93	0·29	2·9	79
1935-36	Chak 132 R. B.	10·7	57·7	12·52	10·22	0·16	1·6	82
1936-37	Chak 126 G. B.	16·1	57·8	12·18	10·28	0·16	1·6	84
Average	..	12·8	57·8	12·10	9·92	0·17	1·7	82

TABLE II

Amount of juice and sucrose, etc., per acre (in tons) and per ton of cane (Lyallpur District)

Variety	Year	Weight per acre				Weight per ton of cane		
		Stripped canes	Juice	Total solids	Sucrose	Juice	Total solids	Sucrose
Co 205	1934-35	14·4	8·90	1·60	1·20	0·62	0·108	0·082
	1935-36	16·0	9·12	1·41	1·73	0·57	0·108	0·088
	1936-37	17·2	10·67	1·91	1·57	0·62	0·112	0·091
	Average	15·9	9·51	1·64	1·50	0·60	0·109	0·087
Co 223	1933-34	18·8	12·04	1·75	1·47	0·64	0·116	0·096
	1934-35	16·7	11·02	1·95	1·61	0·66	0·116	0·096
	1935-36	15·1	9·66	1·81	1·52	0·64	0·120	0·101
	1936-37	23·6	15·58	2·81	2·37	0·66	0·119	0·100
	Average	18·6	12·06	2·08	1·74	0·65	0·118	0·098
Co 213	1933-34	15·6	10·45	1·72	1·42	0·67	0·115	0·094
Co 285	1933-34	31·2	19·34	2·75	2·26	0·62	0·123	0·098
	1934-35	17·5	11·2	2·10	1·70	0·64	0·122	0·096
	1935-36	16·3	9·94	1·90	1·59	0·61	0·117	0·096
	1936-37	21·7	13·45	2·56	2·12	0·62	0·118	0·098
	Average	21·7	13·45	2·33	1·92	0·62	0·120	0·097
Co 290	1934-35	19·9	13·33	2·40	2·04	0·67	0·123	0·102
Ponda	1935-36	26·2	18·34	3·54	3·02	0·70	0·135	0·117
Katha	1933-34	13·7	7·53	1·85	1·52	0·55	0·112	0·093
	1934-35	10·8	6·59	1·41	1·13	0·61	0·125	0·099
	1935-36	10·7	6·21	1·34	1·10	0·58	0·125	0·102
	1936-37	16·1	9·34	1·95	1·64	0·58	0·122	0·103
	Average	12·8	7·42	1·64	1·35	0·58	0·121	0·099

TABLE III

Composition of juice from different varieties of cane—average of four years (Lyallpur District)

Variety of cane	Weight of stripped cane in tons per acre	Per cent on cane of				Glucose ratio	Purity coefficient
		Juice	Total solids	Sucrose	Glucose		
Co 205 . .	15·85	61·1	10·92	8·72	0·42	4·8	80
Co 213 . .	15·6	66·6	11·50	9·40	0·24	2·6	82
Co 223 . .	18·55	65·1	11·79	9·82	0·32	3·6	83
Co 285 . .	21·7	62·2	12·00	9·70	0·18	1·9	81
Co 290 . .	19·9	66·6	12·32	10·20	0·20	2·0	83
Coimbatore (Average)	18·33	64·3	11·71	9·57	0·27	2·8	82
Ponda (Average) .	26·2	69·8	13·5	11·68	0·35	3·0	87
Katha (Average) .	12·8	57·8	12·10	9·92	0·17	1·7	82

TABLE IV

Period of ripeness for different varieties of cane (Lyallpur District)

Year	Varieties of canes						
	Katha	Ponda	Co 205	Co 213	Co 223	Co 285	Co 290
1934-35 . .	26/11 to 10/4	..	20/12 to 4/4	..	27/11 to 25/3	14/12 to 8/4	2/12 to 24/3
1935-36 . .	14/11 to 2/4	30/11 to 12/3	23/11 to 28/3	..	6/12 to 8/4	9/12 to 6/4	..
1936-37 . .	8/11 to 11/4	..	19/11 to 26/3	..	16/11 to 12/4	14/11 to 12/4	16/11 to 12/4
Average period of optimum ripeness	16/11 to 8/4	30/11 to 12/3	1/12 to 30/3	..	26/11 to 5/4	2/12 to 9/4	24/11 to 3/4

N.B.—The figures for 1933-34 are not included as they were abnormal.

TABLE V

Average composition of different varieties of sugarcane during maturity period (Sheikhupura District)

Variety	Year	Weight of stripped cane in tons per acre	Per cent on cane of				Glucose ratio	Purity coefficient
			Juice	Total solids	Sucrose	Glucose		
Co 213 .	1933-34	14·8	64·5	11·50	9·40	0·23	2·5	81
	1934-35	18·1	67·2	11·93	9·90	0·16	1·6	83
	1935-36	23·3	64·9	12·10	9·96	0·14	1·4	83
	1936-37	28·5	67·5	12·80	10·73	0·21	2·0	84
	Average	21·2	66·0	12·08	10·00	0·19	1·9	83
Co 205 .	1934-35	13·3	61·4	10·90	8·34	0·55	6·6	77
	1935-36	26·4	59·2	10·20	8·12	0·51	6·3	79
	1936-37	24·3	62·6	11·26	9·33	0·52	5·6	83
	Average	21·3	61·1	10·79	8·60	0·53	6·2	80
Co 223 .	1933-34	14·9	63·9	11·10	9·33	0·37	4·0	84
	1934-35	15·4	66·5	11·48	9·28	0·41	4·4	87
	1935-36	24·8	64·2	11·72	9·72	0·24	2·9	83
	1936-37	22·8	66·9	11·80	10·17	0·32	3·2	85
	Average	19·5	65·4	11·50	9·63	0·35	3·6	84
Co 285 .	1933-34	23·9	61·8	11·35	9·25	0·22	2·4	82
	1934-35	15·9	63·7	11·36	9·19	0·27	2·9	81
	1935-36	21·8	61·8	11·70	9·79	0·17	1·8	84
	1936-37	25·2	64·2	12·14	10·17	0·22	2·2	84
	Average	21·7	62·9	11·65	9·61	0·22	2·3	83
Co 244 .	1934-35	18·0	67·3	11·67	9·50	0·23	2·4	81
	1935-36	34·9	65·2	12·05	10·40	0·24	2·3	86
	Average	26·4	66·3	11·86	9·95	8·26	2·4	84
Co 313 .	1936-37	27·4	66·4	13·08	11·08	0·19	1·7	85
Ponda .	1936-37	28·9	74·2	13·41	11·95	0·35	2·9	89
Katha .	1933-34	16·8	54·9	11·03	9·08	0·05	0·4	82
	1934-35	16·3	58·4	11·60	9·15	0·20	2·2	79
	1935-36	15·0	54·3	11·37	9·27	0·08	0·8	82
	1936-37	15·9	58·1	11·77	9·89	0·17	1·7	84
	Average	16·0	56·4	11·45	9·35	0·12	1·3	82

TABLE VI

Amount of juice and sucrose, etc., per acre (in tons) and per ton of cane (Sheikhupura District)

Variety	Year	Weight per acre				Weight per ton of cane		
		Stripped canes	Juice	Total solids	Sucrose	Juice	Total solids	Sucrose
Co 205 .	1934-35	13·3	8·08	1·44	1·10	0·61	0·112	0·085
	1935-36	26·4	15·59	2·70	2·15	0·59	0·102	0·081
	1936-37	24·3	15·31	2·74	2·27	0·63	0·113	0·093
	Average	21·3	13·01	2·29	1·84	0·61	0·108	0·086
Co 213 .	1933-34	14·8	9·62	1·72	1·42	0·65	0·115	0·094
	1934-35	18·1	12·13	2·16	1·79	0·67	0·119	0·099
	1935-36	23·3	15·15	2·81	2·33	0·65	0·121	0·100
	1936-37	28·5	19·38	3·62	3·04	0·68	0·128	0·107
	Average	21·2	14·0	2·58	2·15	0·66	0·121	0·100
Co 223 .	1933-34	14·9	9·54	1·75	1·47	0·64	0·111	0·093
	1934-35	15·4	10·32	1·80	1·44	0·67	0·115	0·093
	1935-36	24·8	15·87	2·90	2·41	0·64	0·117	0·097
	1936-37	22·8	15·28	2·68	2·31	0·67	0·118	0·102
	Average	19·5	12·67	2·28	1·9	0·65	0·111	0·096
Co 244 .	1934-35	18·0	12·06	2·10	1·71	0·67	0·117	0·095
	1935-36	34·87	22·66	4·20	3·62	0·65	0·121	0·104
	Average	26·44	17·46	3·15	2·66	0·66	0·119	0·100
Co 285 .	1933-34	23·9	14·82	2·75	2·26	0·62	0·114	0·093
	1934-35	15·9	10·18	1·84	1·49	0·64	0·114	0·092
	1935-36	21·8	13·52	2·55	2·12	0·62	0·117	0·098
	1936-37	25·2	16·13	2·56	2·12	0·64	0·121	0·102
	Average	21·7	13·67	—2·43	2·00	0·63	0·117	0·096
Co 313 .	1936-37	27·4	18·09	3·58	3·04	0·66	0·131	0·111
Ponda .	1936-37	28·9	21·39	3·88	3·45	0·74	0·134	0·120
Katha .	1933-34	16·8	9·24	1·85	1·52	0·55	0·110	0·091
	1934-35	16·3	9·47	1·90	1·49	0·58	0·116	0·092
	1935-36	15·0	8·10	1·71	1·40	0·54	0·114	0·093
	1936-37	15·9	9·22	1·87	1·33	0·58	0·118	0·099
	Average	16·0	8·96	1·83	1·44	0·56	0·115	0·094

TABLE VII

Composition of juice from different varieties of cane—average of four years (Sheikhupura District)

Variety of cane	Weight of stripped cane in tons per acre	Per cent on cane of				Glucose ratio	Purity coefficient
		Juice	Total solids	Sucrose	Glucose		
Co 205 . . .	21·3	61·1	10·79	8·60	0·53	6·2	80
Co 213 . . .	21·2	66·0	12·08	10·00	0·19	1·9	83
Co 223 . . .	19·5	65·4	11·05	9·63	0·35	3·6	87
Co 244 . . .	26·4	66·3	11·86	9·95	0·24	2·4	84
Co 285 . . .	21·7	62·9	11·65	9·61	0·22	2·3	83
Co 313 . . .	27·4	66·4	13·08	11·08	0·19	1·7	85
Average of Coimbatore canes	22·9	64·7	11·75	9·81	0·29	3·0	84
Katha . . .	16·0	56·4	11·45	9·35	0·12	1·3	82
Ponda . . .	28·9	74·2	13·41	11·95	0·35	2·9	89

TABLE VIII

Period of ripeness for different varieties of cane (Sheikhupura District)

Year	Varieties of cane							
	Katha	Ponda	Co 205	Co 213	Co 223	Co 244	Co 285	Co 313
1934-35 .	26/11 to 6/4	..	27/11 to 19/3	1/1 to 29/3	28/11 to 5/3	28/11 to 4/4	29/11 to 5/4	..
1935-36 .	21/11 to 5/4	..	26/11 to 6/4	3/12 to 2/4	27/11 to 7/4	21/11 to 30/3	19/11 to 7/4	..
1936-37 .	10/11 to 12/4	20/11 to 16/4	6/11 to 18/4	12/11 to 17/4	12/11 to 10/4	.. ..	12/11 to 10/4	5/11 to 7/4
Average .	19/11 to 8/4	20/11 to 16/4	20/11 to 4/4	6/12 to 6/4	22/11 to 23/3	25/11 to 2/4	20/11 to 6/4	5/11 to 7/4

N.B.—The figures for 1933-34 are not included as they were abnormal.

TABLE IX

Average composition of different varieties of sugarcane during maturity period (Lahore District)

Variety	Year	Weight of stripped cane in tons per acre	Per cent on cane of				Glucose ratio	Purity coefficient
			Juice	Total solids	Sucrose	Glucose		
Katha .	1933-34	11·0	53·1	10·30	8·40	0·21	2·5	82
Kahu .	1935-36	6·4	65·1	12·86	10·84	0·35	3·2	84
Ponda .	1933-34	27·9	71·5	12·28	10·98	0·51	4·6	89
	1934-35	18·7	70·8	12·29	11·03	0·39	3·5	90
	1935-36	41·5	72·2	12·81	11·16	0·48	4·3	87
	1936-37	32·3	71·5	12·78	11·32	0·36	3·2	89
	Average	28·8	71·4	12·47	11·08	0·45	4·1	89
Co 205 .	1933-34	27·3	57·9	10·40	8·60	0·48	5·6	83
	1934-35	14·7	59·2	10·05	7·95	0·79	9·9	79
	1935-36	12·1	58·3	10·84	9·11	0·38	4·2	84
	Average	18·0	58·5	10·43	8·55	0·55	6·6	82
Co 223 .	1933-34	17·6	64·4	11·20	9·68	0·45	4·7	86
	1934-35	18·2	65·8	10·76	9·08	0·51	5·6	84
	1935-36	19·8	64·9	11·65	9·92	0·47	4·7	85
	1936-37	16·7	66·7	11·48	9·90	0·38	3·8	86
	Average	17·9	65·6	11·31	9·69	0·44	4·5	86
Co 285 .	1933-34	24·7	59·8	11·40	9·80	0·35	3·6	86
	1934-35	25·3	61·7	11·17	9·47	0·30	3·2	85
	1935-36	19·1	63·3	12·10	10·32	0·24	2·3	85
	1936-37	18·5	64·2	12·09	10·33	0·23	2·2	86
	Average	20·1	63·3	11·84	10·09	0·25	2·5	85
Co 290 .	1933-34	28·8	66·4	12·10	10·40	0·35	3·4	86
	1934-35	15·2	69·0	12·19	10·26	0·45	4·6	84
	1935-36	6·0	64·8	13·04	11·45	0·21	1·8	88
	1936-37	7·0	67·2	12·33	10·89	0·25	2·3	88
	Average	12·8	66·9	12·40	10·78	0·30	2·8	87

TABLE X

Amount of juice and sucrose, etc., per acre (in tons) and per ton of cane (Lahore District)

Variety	Year	Weight per acre				Weight per ton of cane		
		Stripped canes	Juice	Total solids	Sucrose	Juice	Total solids	Sucrose
Katha .	1933-34	11·0	5·84	1·13	0·92	0·53	0·103	0·084
Kahu .	1935-36	6·4	4·17	0·82	0·69	0·65	0·129	0·108
Ponda .	1933-34	27·9	20·0	3·43	3·07	0·72	0·123	0·110
	1934-35	18·7	13·2	2·30	2·06	0·71	0·123	0·110
	1935-36	41·5	30·0	5·32	4·63	0·72	0·128	0·112
	1936-37	32·3	23·1	4·13	3·66	0·72	0·128	0·114
	Average	28·8	20·6	3·59	3·19	0·71	0·125	0·111
Co 205 .	1933-34	27·3	15·8	2·84	2·35	0·58	0·104	0·086
	1934-35	14·7	8·7	1·48	1·17	0·59	0·101	0·080
	1935-36	12·1	7·06	1·31	1·10	0·58	0·108	0·091
	Average	18·0	10·5	1·88	1·54	0·59	0·104	0·086
Co 223 .	1933-34	17·6	11·3	1·97	1·70	0·64	0·112	0·097
	1934-35	18·2	12·0	1·96	1·66	0·66	0·108	0·091
	1935-36	19·8	12·9	2·31	1·96	0·65	0·117	0·099
	1936-37	16·7	11·1	1·92	1·65	0·67	0·115	0·099
	Average	17·9	11·7	2·02	1·73	0·66	0·113	0·097
Co 285 .	1933-34	24·7	14·8	2·82	2·42	0·60	0·114	0·098
	1934-35	25·2	15·6	2·83	2·40	0·62	0·112	0·095
	1935-36	19·1	12·1	2·31	1·97	0·63	0·121	0·103
	1936-37	18·5	11·9	2·24	1·91	0·64	0·121	0·103
	Average	20·1	12·7	2·38	2·03	0·63	0·118	0·101
Co 290 .	1933-34	28·8	19·1	3·49	3·00	0·66	0·121	0·104
	1934-35	15·2	10·5	1·85	1·56	0·69	0·122	0·103
	1935-36	6·0	3·89	0·78	0·69	0·65	0·130	0·115
	1936-37	7·0	4·70	0·86	0·76	0·67	0·123	0·109
	Average.	12·8	8·56	1·59	1·38	0·67	0·124	0·108
Co 312 .	1936-37	7·0	4·61	0·84	0·75	0·66	0·119	0·107

TABLE XI

Composition of juice from different varieties of sugarcane—average of four years (Lahore District)

Variety of cane	Weight of stripped cane in tons per acre	Per cent on cane of				Glucose ratio	Purity coefficient
		Juice	Total solids	Sucrose	Glucose		
Katha . . .	11·0	53·1	10·3	8·4	0·21	2·5	82
Kahu . . .	6·4	65·1	12·86	10·84	0·35	3·2	84
Ponda . . .	28·8	71·4	12·47	11·08	0·45	4·1	89
Co 205 . . .	18·0	58·5	10·43	8·55	0·55	6·6	82
Co 223 . . .	17·9	65·6	11·31	9·69	0·44	4·5	86
Co 285 . . .	20·1	63·3	11·84	10·09	0·25	2·2	85
Co 290 . . .	12·8	66·9	12·40	10·78	0·30	2·8	87
Co 312 . . .	7·0	65·8	11·97	10·68	0·18	1·7	89
Average for Coimbatore canes.	15·2	64·0	11·59	9·96	0·34	3·4	86
Average for Coimbatore canes (excluding Co. 290 & 312).	18·7	62·5	11·19	9·44	0·41	4·3	84

TABLE XII

Period of ripeness for different varieties of sugarcane (Lahore District)

Year	Varieties of cane							
	Katha	Kahu	Ponda	Co 205	Co 223	Co 285	Co 290	Co 312
1933-34 .	5/12 to 19/3	..	6/12 to 13/3	12/12 to 8/4	8/12 to 26/3	12/12 to 8/4	12/12 to 26/3	..
1934-35 .	..	..	9/12 to 21/2	22/12 to 13/4	5/12 to 22/3	18/12 to 1/4	22/12 to 16/2	..
1935-36 .	..	24/11 to 11/3	28/11 to 24/2	7/1 to 14/4	1/12 to 22/3	4/12 to 6/4	15/12 to 17/3	.. ..
1936-37 .	..	..	20/11 to 17/3	..	24/11 to 15/3	13/11 to 9/4	10/11 to 7/3	28/11 to 26/3
Average period of optimum ripeness.	5/12 to 19/3	24/11 to 11/3	1/12 to 5/3	24/12 to 12/4	5/12 to 23/3	4/12 to 6/4	7/12 to 10/3	28/11 to 26/3

TABLE XIII

Average composition of different varieties of sugarcane during maturity period (Montgomery District)

Variety	Year	Weight of stripped cane in tons per acre	Per cent on cane of				Glucose ratio	Purity co-efficient
			Juice	Total solids	Sucrose	Glucose		
Katha .	1934-35	16·1	57·3	12·14	9·94	0·10	0·1	82
	1935-36	12·2	57·5	12·45	10·19	0·19	1·8	82
	1936-37	11·4	59·5	12·21	9·79	0·34	3·5	80
	Average	13·0	58·0	12·31	10·03	0·20	1·8	81
Ponda .	1934-35	22·1	71·1	12·81	11·10	0·51	4·7	86
Co. 285 .	1933-34	20·3	60·5	10·98	9·32	0·23	2·5	85
	1934-35	20·9	63·2	11·41	9·52	0·25	2·6	84
	1935-36	23·9	62·4	12·02	10·24	0·17	1·7	85
	1936-37	24·2	64·3	12·15	10·41	0·18	1·7	86
	Average	21·7	60·4	11·27	9·57	0·20	2·1	85
Co. 223 .	1933-34	17·5	63·8	10·96	9·34	0·40	4·3	85
	1934-35	17·3	65·2	10·87	9·13	0·37	4·1	84
	1935-36	22·0	64·9	11·72	10·01	0·29	2·9	85
	1936-37	17·1	66·6	11·66	10·05	0·27	2·7	86
	Average	18·6	65·3	11·36	9·70	0·33	3·4	85
Co. 205 .	1933-34	19·9	59·1	10·30	8·66	0·41	4·7	84
	1934-35	18·8	62·3	10·39	8·37	0·50	6·0	81
	1935-36	20·5	60·6	11·57	9·30	0·43	4·6	81
	Average	19·9	59·8	10·59	8·77	0·43	4·9	83
Co. 270 .	1933-34	15·8	62·6	11·60	10·10	0·30	3·0	87
Co. 290 .	1933-34	18·3	64·9	10·60	8·90	0·83	5·6	84
	1934-35	12·2	67·2	12·09	10·39	0·25	2·4	86
	1935-36	18·3	69·4	13·20	11·77	0·16	1·4	89
	1936-37	21·6	69·7	12·05	10·31	0·22	2·1	86
	Average	16·6	67·9	12·03	10·34	0·27	2·6	86

TABLE XIII—*contd.*

Variety	Year	Weight of stripped cane in tons per acre	Juice	Per cent on cane of			Glucose ratio	Purity co-efficient
				Total solids	Sucrose	Glucose		
Co. 300 .	1934-35	12·7	67·9	12·29	10·60	0·21	2·1	86
	1935-36	27·1	65·9	13·12	11·65	0·09	0·7	89
	1936-37	21·3	66·2	11·76	10·00	0·14	1·4	85
	Average	18·5	67·0	12·36	10·72	0·16	1·6	87
Co. 301 .	1934-35	18·1	67·2	11·82	9·98	0.23	2·3	85
	1935-36	27·1	65·9	13·12	11·65	0·09	0·1	89
	1936-37	21·4	69·1	11·93	9·96	0·20	2·0	84
	Average	22·2	67·4	12·29	10·53	0·17	1·8	86
Co. 312 .	1934-35	16·8	68·2	11·42	9·72	0·24	2·5	85
	1935-36	27·9	66·2	12·50	11·04	0·19	1·4	88
	1936-37	30·4	67·7	11·31	9·42	0·20	2·2	84
	Average	25·8	67·4	11·64	9·97	0·20	2·0	86
Co. 313 .	1934-35 .	10·0	64·9	11·89	10·14	0·18	1·9	85
	1935-36	26·3	67·5	13·23	11·70	0·15	1·3	88
	1936-37	30·8	67·8	13·69	11·87	0·16	1·3	87
	Average	19·3	66·3	12·68	10·96	0·17	1·4	86
Co. 213 .	1933-34	11·0	60·3	11·00	9·20	0·17	1·8	84
	1935-36	20·9	65·9	12·38	10·71	0·16	1·5	87
	1936-37	27·0	67·3	12·87	11·15	0·15	1·3	87
	Average	19·9	64·8	12·16	10·45	0·16	1·5	86
Co .210 .	1935-36	11·7	66·6	11·96	10·00	0·16	1·6	84

TABLE XIV

Amount of juice and sucrose, etc., per acre (in tons) and per ton of cane (Montgomery District)

Variety	Year	Weight per acre				Weight per ton of cane		
		Stripped canes	Juice	Total solids	Sucrose	Juice	Total solids	Sucrose
Katha	1934-35	16·1	9·2	1·95	1·60	0·51	0·121	0·099
	1935-36	12·2	7·0	1·52	1·24	0·58	0·125	0·102
	1936-37	11·4	6·8	1·39	1·12	0·60	0·122	0·098
	Average	13·0	7·5	1·60	1·30	0·58	0·123	0·100
Ponda	1934-35	22·1	15·7	2·85	2·45	0·71	0·129	0·111
Co 285	1933-34	20·3	12·3	2·23	1·89	0·61	0·110	0·093
	1934-35	20·9	13·2	2·38	1·99	0·63	0·114	0·095
	1935-36	23·9	14·9	2·87	2·45	0·62	0·120	0·102
	1936-37	24·2	15·6	2·94	2·52	0·64	0·122	0·104
	Average	21·7	13·1	2·45	2·08	0·60	0·113	0·096
Co 223	1933-34	17·5	11·2	1·92	1·64	0·64	0·110	0·093
	1934-35	17·3	11·3	1·88	1·58	0·65	0·109	0·091
	1935-36	22·0	14·3	2·58	2·20	0·65	0·117	0·100
	1936-37	17·1	11·4	1·99	1·72	0·67	0·124	0·108
	Average	18·6	12·2	2·11	1·80	0·65	0·114	0·097
Co 205	1933-34	19·9	11·8	2·05	1·72	0·59	0·103	0·087
	1934-35	18·8	11·7	1·95	1·57	0·62	0·104	0·084
	1935-36	20·5	12·4	2·37	1·91	0·61	0·116	0·093
	Average	19·9	11·9	2·11	1·75	0·60	0·106	0·088
Co 270	1933-34	15·8	9·9	1·83	1·60	0·63	0·116	0·101
Co 290	1933-34	18·3	11·90	1·94	1·63	0·65	0·106	0·089
	1934-35	12·2	7·83	1·41	1·21	0·67	0·121	0·104
	1935-36	18·3	12·7	2·42	2·15	0·69	0·132	0·118
	1936-37	21·6	15·1	2·60	2·23	0·70	0·121	0·103
	Average	16·6	11·3	2·00	1·72	0·68	0·120	0·103
Co 300	1934-35	12·7	8·6	1·56	1·35	0·68	0·123	0·106
	1935-36	27·1	17·9	3·56	3·16	0·66	0·131	0·117
	1936-37	21·3	14·1	2·50	2·13	0·66	0·118	0·100
	Average	18·5	12·4	2·29	1·98	0·66	0·124	0·107
Co 301	1934-35	18·1	12·2	2·14	1·81	0·67	0·118	0·100
	1935-36	27·1	17·9	3·56	3·16	0·66	0·131	0·117
	1936-37	21·4	14·8	2·55	2·13	0·69	0·119	0·100
	Average	22·4	15·0	2·73	2·34	0·67	0·123	0·105

TABLE XIV—*contd.*

Variety	Year	Weight per acre				Weight per ton of cane		
		Stripped canes	Juice	Total solids	Sucrose	Juice	Total solids	Sucrose
Co 312	1934-35	16·8	11·5	1·92	1·63	0·68	0·114	0·097
	1935-36	27·9	18·5	3·49	3·08	0·66	0·125	0·110
	1936-37	30·4	20·6	3·44	2·86	0·68	0·113	0·094
	Average	25·8	17·4	3·00	2·57	0·67	0·116	0·100
Co 313	1934-35	10·0	6·5	1·19	1·01	0·65	0·119	0·101
	1935-36	26·3	17·8	3·48	3·08	0·68	0·132	0·117
	1936-37	30·8	20·9	4·22	3·66	0·68	0·137	0·119
	Average.	19·3	12·8	2·45	2·12	0·66	0·127	0·110
Co 213	1933-34	11·0	6·6	1·21	1·01	0·60	0·110	0·092
	1935-36	20·9	13·8	2·59	2·24	0·67	0·124	0·107
	1936-37	27·0	18·2	3·47	3·01	0·67	0·129	0·112
	Average	19·9	12·9	2·42	2·08	0·65	0·122	0·105
Co 210	1935-36	11·7	7·8	1·40	1·17	0·67	0·120	0·100

TABLE XV

Composition of juice from different varieties of sugarcane—average of four years (Montgomery District)

Variety of cane	Weight of stripped cane in tons per acre	Per cent on cane of				Glucose ratio	Purity coefficient
		Juice	Total solids	Sucrose	Glucose		
Katha . . .	13·0	58·0	12·31	10·03	0·20	1·8	82
Ponda . . .	22·1	71·1	12·89	11·10	0·51	4·7	86
Co 205 . . .	19·9	59·8	10·59	8·77	0·43	4·9	83
Co 210 . . .	11·7	66·5	11·96	10·00	0·16	1·6	84
Co 223 . . .	18·6	65·3	11·36	9·70	0·33	3·4	85
Co 213 . . .	19·9	64·8	12·16	10·45	0·16	1·5	86
Co 285 . . .	21·7	60·4	11·27	9·57	0·20	2·1	85
Co 290 . . .	16·6	67·9	12·03	10·34	0·27	2·6	86
Co 300 . . .	18·5	67·0	12·36	10·72	0·16	1·6	87
Co 301 . . .	22·2	67·4	12·29	10·53	0·17	1·8	86
Co 312 . . .	25·8	67·4	11·64	9·97	0·20	2·0	86
Co 313 . . .	19·3	66·3	12·68	10·96	0·17	1·4	86
Co 270 . . .	15·8	62·6	11·60	10·10	0·30	3·0	87
Average of Coimbatore canes.	19·1	64·9	11·81	10·11	0·23	2·3	86

TABLE XVI

Period of optimum ripeness for different varieties of sugarcane (Montgomery District)

Year	Katha	Ponda	Co 205	Co 223	Co 285	Co 290	Co 312	Co 213	Co 210	Co 300	Co 301	Co 313	Co 270
1933-34	...	...	15/12 to 2/4	10/12 to 29/3	12/12 to 5/4	3/12 to 4/3	...	19/12 to 20/3	...	...	...	...	14/12 to 11/4
1934-35	23/11 to 1/3	30/11 to 15/2	29/11 to 30/3	6/12 to 23/3	11/12 to 16/4	11/12 to 26/3	4/12 to 2/4	...	...	27/11 to 16/3	29/12 to 20/4	27/11 to 9/4	...
1935-36	17/11 to 9/4	...	15/12 to 28/3	30/11 to 4/4	17/11 to 11/4	3/12 to 13/4	5/12 to 9/4	25/11 to 15/4	3/12 to 14/4	16/11 to 13/4	1/11 to 13/4	1/11 to 14/4	...
1936-37	24/10 to 17/2	...	...	16/11 to 31/3	10/11 to 2/4	24/11 to 22/3	25/11 to 2/4	24/12 to 7/4	...	5/11 to 1/4	5/11 to 1/4	28/10 to 20/3	...
Average period of optimum ripeness	11/11 to 10/3	30/11 to 15/12	10/12 to 30/3	1/12 to 29/3	28/11 to 9/4	3/12 to 24/3	1/12 to 4/4	13/12 to 4/4	3/12 to 14/4	16/11 to 10/4	22/11 to 11/4	9/11 to 1/4	14/12 to 11/4

TABLE XVII

Average composition of different varieties of sugarcane during maturity period (Multan District)

Variety	Year	Weight of stripped cane in tons per acre	Per cent on cane of				Glucose ratio	Purity coefficient
			Juice	Total solids	Sucrose	Glucose		
Katha .	1934-35	17·6	56·3	11·98	10·20	0·11	1·1	86
	1935-36	14·7	57·4	12·17	10·45	0·07	0·7	86
	1936-37	11·0	56·8	11·83	9·68	0·14	1·5	84
Ponda .	1933-34	21·3	67·3	11·70	10·10	0·27	2·7	86
	1934-35	16·1	69·8	12·10	10·66	0·36	3·5	88
	Average	18·7	68·6	11·90	10·38	0·32	3·1	87
Co 205 .	1933-34	25·2	60·2	10·50	8·60	0·32	3·7	82
	1934-35	17·0	59·0	11·09	9·23	0·46	5·0	83
	1935-36	19·9	60·8	12·07	10·31	0·10	3·1	85
	Average	19·8	59·7	11·19	9·34	0·33	4·2	83
Co 213 .	1933-34	12·9	62·2	9·90	8·40	0·26	3·1	85
	1934-35	8·8	63·6	11·00	9·40	0·06	0·7	86
	Average	10·9	62·9	10·45	8·90	0·16	1·9	86
Co 223 .	1933-34	25·3	64·2	10·25	9·05	0·42	4·7	84
	1934-35	19·7	64·5	10·78	9·14	0·27	3·0	85
	1935-36	20·0	65·1	12·08	10·48	0·18	1·8	87
	1936-37	19·8	66·7	12·09	10·67	0·21	2·0	88
	Average	21·2	64·9	11·26	9·64	0·28	2·9	86
Co 281 .	1933-34	12·4	64·5	11·60	10·00	0·30	3·0	87
	1934-35	9·5	66·5	12·70	10·63	0·10	1·0	87
	Average	11·4	65·5	11·76	10·21	0·23	2·4	87
Co 285 .	1933-34	27·1	59·9	10·95	9·40	0·13	1·0	86
	1934-35	19·7	62·9	11·28	9·17	0·13	1·4	81
	1935-36	25·9	62·1	12·39	10·41	0·10	0·9	84
	1936-37	18·7	63·5	11·63	9·46	0·14	1·4	81
	Average	22·8	62·2	11·62	9·65	0·12	1·3	83
Co 290 .	1933-34	25·2	64·5	11·20	9·65	0·28	2·9	86
	1934-35	20·9	69·1	11·86	9·87	0·26	2·7	83
	1935-36	24·0	66·0	12·96	11·06	0·18	1·6	85
	1936-37	19·0	68·6	12·83	11·27	0·15	1·3	88
	Average	22·9	64·5	12·01	10·30	0·23	2·3	87
Co 312 .	1933-34	17·6	65·1	10·40	8·80	0·32	3·8	85
	1935-36	25·6	67·5	13·09	11·45	0·12	1·1	88
	1936-37	30·2	67·5	12·82	11·18	0·13	1·2	87
	Average	24·5	66·7	12·10	10·48	0·19	2·0	86

TABLE XVIII

Amount of juice and sucrose, etc., per acre (in tons) and per ton of cane (Multan District)

Variety	Year	Weight per acre				Weight per ton of cane		
		Stripped canes	Juice	Total solids	Sucrose	Juice	Total solids	Sucrose
Katha .	1934-35	17·6	9·91	2·11	1·80	0·56	0·120	0·102
	1935-36	14·7	8·44	1·79	1·54	0·57	0·122	0·105
	1936-37	11·0	6·25	1·27	1·07	0·57	0·115	0·097
	Average	14·4	8·18	1·71	1·45	0·57	0·119	0·101
Ponda .	1933-34	21·3	14·3	2·49	2·15	0·67	0·117	0·101
	1934-35	16·1	11·2	1·95	1·72	0·70	0·121	0·107
	Average	18·7	12·8	2·23	1·94	0·69	0·119	0·104
Co 205 .	1933-34	25·2	15·2	2·65	2·17	0·60	0·105	0·086
	1934-35	17·0	10·0	1·89	1·57	0·59	0·111	0·092
	1935-36	19·9	12·1	2·40	2·05	0·61	0·121	0·103
	Average	19·8	11·8	2·22	1·85	0·60	0·112	0·093
Co 223 .	1933-34	25·3	16·2	2·72	2·29	0·64	0·108	0·091
	1934-35	19·7	12·7	2·12	1·80	0·65	0·108	0·091
	1935-36	20·0	13·0	2·42	2·10	0·65	0·121	0·105
	1936-37	19·8	13·2	2·39	2·11	0·67	0·121	0·107
	Average	21·2	13·8	2·39	2·04	0·65	0·113	0·096
Co 285 .	1933-34	27·1	16·2	2·97	2·55	0·60	0·110	0·094
	1934-35	19·7	12·4	2·22	1·81	0·63	0·113	0·092
	1935-36	25·9	16·1	3·21	2·70	0·62	0·124	0·104
	1936-37	18·7	11·9	2·18	1·77	0·64	0·116	0·095
	Average	22·8	14·2	2·65	2·20	0·62	0·116	0·097
Co 281 .	1933-34	12·4	8·0	1·44	1·24	0·65	0·116	0·100
	1934-35	9·5	6·32	1·16	1·01	0·67	0·122	0·106
	Average	11·4	7·42	1·34	1·16	0·65	0·118	0·102
Co 290 .	1933-34	25·2	16·3	2·82	2·43	0·65	0·112	0·097
	1934-35	20·9	14·4	2·48	2·06	0·69	0·119	0·099
	1935-36	24·0	15·8	3·11	2·66	0·66	0·130	0·110
	1936-37	19·0	13·0	2·44	2·14	0·69	0·128	0·113
	Average	22·9	14·8	2·75	2·36	0·65	0·120	0·103
Co 312 .	1933-34	17·6	11·7	1·83	1·55	0·65	0·104	0·088
	1935-36	25·6	17·3	3·35	2·93	0·68	0·131	0·115
	1936-37	30·2	20·4	3·87	3·38	0·68	0·128	0·112
	Average	24·5	16·3	2·97	2·57	0·67	0·121	0·105
Co 213 .	1933-34	12·9	8·02	1·28	1·08	0·062	0·099	0·084
	1934-35	8·8	5·60	0·097	0·083	0·064	0·110	0·094
	Average	10·9	6·86	1·14	0·097	0·063	0·105	0·089

TABLE XIX

Composition of juice from different varieties of cane—average of four years (Multan District)

Variety of cane	Weight of stripped cane in tons per acre	Per cent on cane of				Glucose ratio	Purity coefficient
		Juice	Total solids	Sucrose	Glucose		
Katha . . .	14·4	56·8	11·89	10·11	0·11	1·1	85
Ponda . . .	18·7	68·6	11·90	10·38	0·32	3·1	87
Co 205 . . .	19·8	59·7	11·19	9·34	0·33	4·2	83
Co 223 . . .	21·2	64·9	11·26	9·64	0·28	2·9	86
Co 285 . . .	22·8	62·2	11·62	9·65	0·12	1·3	83
Co 290 . . .	22·9	64·5	12·01	10·30	0·23	2·3	86
Co 281 . . .	11·4	65·1	11·76	10·21	0·23	2·4	87
Co 312 . . .	24·5	66·7	12·10	10·48	0·19	2·0	86
Co 213 . . .	10·9	62·9	10·45	8·90	0·16	1·9	86
Average of Coimbatore canes.	19·1	63·7	11·48	9·79	0·22	2·3	85

TABLE XX

Period of ripeness for different varieties of sugarcane (Multan District)

Year	Varieties of cane								
	Katha	Ponda	Co 205	Co 223	Co 285	Co 290	Co 312	Co 281	Co 213
1933-34	...	11/12 to 6/2	6/12 to 23/3	9/12 to 1/4	16/12 to 25/3	11/12 to 8/4	6/12 to 18/3	6/12 to 8/3	...
1934-35	28/11 to 17/4	11/12 to 19/3	22/12 to 6/4	18/12 to 18/3	9/12 to 2/4	27/11 to 5/3	...	3/12 to 8/4	3/12 to 11/3
1935-36	19/11 to 1/4	...	8/1 to 1/4	1/12 to 25/3	1/12 to 8/4	26/11 to 24/3	26/11 to 7/4	...	...
1936-37	24/10 to 4/4	...	...	23/11 to 3/4	8/11 to 4/4	23/11 to 3/4	23/11 to 3/4	...	...
Average period of optimum ripeness.	13/11 to 7/4	11/12 to 26/2	22/12 to 31/3	5/12 to 27/3	1/12 to 2/4	29/11 to 26/3	28/11 to 30/3	5/12 to 24/3	3/12 to 11/3

TABLE XXI

Locality	Stripped cane in tons per acre		Sucrose in tons per acre	
	Local	Coimbatore	Local	Coimbatore
Rohtak-Karnal . . .	24·6	26·7	1·83	2·35
Jullundur-Hoshiarpur . .	17·3	24·1	1·57	2·62
Gurdaspur-Sialkot . .	11·2	19·6	1·04	1·95
Lahore	8·7	18·7	0·80	1·76
Montgomery . . .	13·0	19·1	1·30	1·93
Multan	14·4	19·1	1·45	1·75
Lyallpur	12·8	18·3	1·35	1·72
Sheikhupura . . .	16·0	22·9	1·44	2·36

TABLE XXII

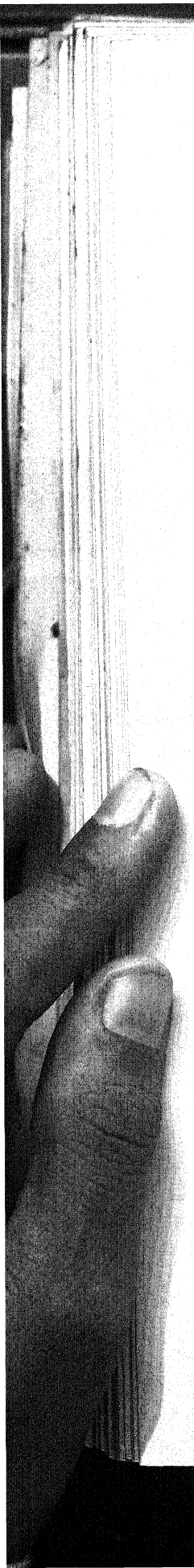
Period of ripeness and length of crushing season (in days) for different varieties of cane in different parts of the Punjab

Varieties	Sheikhupura	Lahore	Lyallpur	Montgomery	Multan	Jullundur	Gurdaspur	Rohtak and Karnal
Katha	19/11 to 8/4 (141 days)	5/12 to 19/3 (105 days)	16/11 to 8/4 (145 days)	11/11 to 10/3 (120 days)	13/11 to 7/4 (146 days)	21/11 to 8/3 (108 days)	...	...
Ponda	20/11 to 16/4 (148 days)	1/12 to 5/3 (95 days)	30/11 to 12/3 (103 days)	30/11 to 15/2 (78 days)	11/12 to 26/2 (78 days)	...	...	...
Co 205	20/11 to 4/4 (135 days)	24/12 to 12/4 (110 days)	1/12 to 30/3 (120 days)	10/12 to 30/3 (111 days)	22/12 to 31/3 (100 days)	10/2 to 10/4 (60 days)	31/1 to 2/4 (61 days)	10/1 to 2/4 (83 days)
Co 223	22/11 to 23/3 (122 days)	5/12 to 23/3 (109 days)	26/11 to 5/4 (131 days)	1/12 to 29/3 (119 days)	5/12 to 27/3 (119 days)	15/1 to 21/3 65 (days)	3/1 to 30/3 (87 days)	14/1 to 23/3 (69 days)
Co 285	20/11 to 6/4 (128 days)	4/12 to 6/4 (124 days)	2/12 to 9/4 (129 days)	28/11 to 9/4 (133 days)	1/12 to 2/4 (123 days)	20/1 to 12/4 (83 days)	30/12 to 10/4 (102 days)	...
Co 290	...	7/12 to 10/3 (94 days)	...	3/12 to 24/3 (112 days)	29/11 to 26/3 (118 days)	7/1 to 13/4 (97 days)	22/12 to 28/3 (97 days)	...
Co 312	...	28/11 to 26/3 (119 days)	...	1/12 to 4/4 (125 days)	28/11 to 30/3 (123 days)	...	...	...
Co 281	...	...	...	...	5/12 to 24/3 (110 days)	24/12 to 11/4 (109 days)	...	...
Co 213	6/12 to 6/4 (122 days)	...	8/1 to 7/4 (90 days)	13/12 to 4/4 (113 days)	3/12 to 11/3 (99 days)	22/1 to 13/4 (84 days)	8/1 to 1/4 (83 days)	31/1 to 28/3 (58 days)
Co 210	...	...	...	3/12 to 14/4 (133 days)	...	...	...	...
Co 300	...	...	...	16/11 to 10/4 (146 days)	...	...	15/12 to 20/2 (68 days)	...
Co 301	...	...	...	22/11 to 11/4 (141 days)	...	...	...	...
Co 313	5/11 to 7/4 (154 days)	...	...	9/11 to 1/4 (144 days)	...	...	...	...
Co 270	...	...	...	14/12 to 11/4 119 days)	...	...	...	...
Co 244	25/11 to 2/4 (129 days)	...	...	...	...	...	...	...

TABLE XXIII

Area (in acres) under sugarcane in the Punjab

District	1933-34	1934-35	1935-36	1936-37
Hissar	1,077	758	1,272	2,678
Rohtak	32,053	31,412	29,575	38,167
Gurgaon	7,312	9,805	11,718	13,921
Karnal	25,795	25,095	30,463	37,929
Ambala	22,370	24,490	28,285	29,832
Simla	..	..	..	..
Kangra	4,301	4,317	4,535	5,284
Hoshiarpur	28,461	27,738	28,216	30,523
Jullundur	30,068	34,424	35,252	39,252
Ludhiana	14,182	14,777	17,314	21,020
Ferozepur	7,433	6,097	9,024	15,812
Lahore	18,583	16,617	19,813	28,220
Amritsar	28,724	30,032	28,732	29,989
Gurdaspur	54,220	55,079	53,807	63,934
Sialkot	37,258	37,606	36,386	43,019
Gujranwala	27,519	28,010	27,058	29,888
Sheikhupura	21,790	18,270	16,440	20,493
Gujrat	15,775	15,438	11,459	12,500
Shahpur	9,538	8,266	6,860	7,168
Jhelum	236	311	226	207
Rawalpindi	98	97	114	97
Attock	497	517	721	990
Mianwali	95	47	71	130
Montgomery	13,545	11,378	12,913	14,498
Lyallpur	41,675	42,635	40,918	41,281
Jhang	3,699	4,378	3,788	3,292
Multan	12,178	9,752	12,419	14,892
Muzaffargarh	7,304	4,958	6,631	9,123
D. G. Khan	205	138	190	419
GRAND TOTAL	4,65,991	4,62,442	4,74,200	5,54,348



PROBLEMS OF SUGARCANE PHYSIOLOGY IN THE DECCAN-CANAL TRACT

I. GERMINATION

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(With four text-figures)

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I. Introduction

A vigorous and uniform germination is very important from the standpoint of the practical agriculturist, who, in the absence of the knowledge of the conditions for good germination, always uses a large seed rate as a precaution against low germination, and thus the material, which could be utilised for either *gul* or sugar making, is wasted to a very great extent. In the Deccan-Canal tract, the common practice was to use such a large number as 54,000 eye-buds per acre. Some field experimental work was, therefore, carried out at Manjri on the proper method of planting, and as a result the Bombay Department of Agriculture [Anonymous, 1915] has recommended about 50 per cent reduction in the number of setts required for the purpose. The authors during their first year's physiological investigations, however, observed not only a great deal of variability in the germinative capacity of the

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eye-buds from variety to variety, but even in the same variety if planted at different places and different seasons. This required thorough investigations and the work described in this paper forms a part of these studies.

II. Historical

It is not proposed to go into a detailed historical survey of all the literature on this subject. Only salient features will be touched. Tempany [1924] observes that the young shoot is entirely dependent on the parent stem for nutriment until the fifth week and is capable of drawing from the reserves of food in the portions of the stem, both above and below the node to which the particular eye-bud is attached. Bonazii [1928], on the other hand, states that as the vascular bundles entering a bud are traceable to the internodes immediately below the bud, the young shoot can get its nourishment from that internode only and therefore the lower bud of the piece would find at its disposal only a smaller quantity of reserve material than the others. In the case of two eye-bud setts there is thus a tendency according to him for the upper bud to develop more than the other. Venkatraman [1926] found that removing the reserve tissue from the immediate vicinity of ungerminated cane buds (either by splitting, sectioning, ringing or by removing pith in a seed piece) caused these to germinate much more slowly than untreated buds, showing thereby that germination is to a certain extent dependent upon the presence of reserve material in the seed piece. According to him the ordinary three-budded cane setts contain enough plant food to keep the plant going for at least six weeks.

As regards the chemical processes involved during germination, Tempany [1924] maintains that the storage of sucrose in the stem of the sugarcane is a definite provision for the propagation of the plant by means of shoots from the eye-buds, and in case any inversion takes place as a prelude to metabolism, the reducing sugars formed are used up as fast as they are produced without resulting in any increase in its content. Bonazii [1928] finds that the germinability of cane buds is directly correlated with water and glucose content of the meristematic tissues of the eye-buds, whereas it bears an inverse correlation to the osmotic pressure of the juices circulating in these. According to him the gradients of germination are concomitant with and resulting from accumulation in the juices of the meristematic tissues of high osmotic pressure. From these conclusions he recommends that the selection of canes for vegetative growth is to be done on the basis of individual canes and not on the basis of buds and that seed pieces from the 'upper mature' portions of a cane which have a more precocious development should therefore obtain preference in field planting. Venkatraman [1929] has found quick and vigorous germination in the unripe portions of the cane and he therefore recommends the planting of unripe canes or unripe portions of canes to get good germination. Evans [1936] has shown a correlation of $-0{\cdot}62 \pm 0{\cdot}097$ between the reducing sugar content and the number of days required for germination ; indicating the desirability of the selection of theplanting material with a high content of reducing sugar.

Methods of soaking setts in water and other chemicals before planting have been tried with success in Porto Rico [May, 1927], Mauritius [Evans, 1936] and India [Khanna, 1934]. Among the various treatments soaking in lime or lime and magnesia has given significantly higher yields. This is explained by the fact that cuttings absorb more water from a lime solution than from pure water.

III. Methods

Botanical

(*1*) *Total height.*—This is measured by the Kamerling's method [1904] which consists of taking the height from the ground level up to the highest visible transverse leaf mark. A flat headed peg is buried to the level of the ground from which all periodical measurements are done in order to avoid the effect of fluctuations in the ground level due to irrigation, interculturing, etc.

(*2*) *Height of millable canes.*—In the flowering varieties like P O J 2878, there is a rapid elongation of the shoot mainly serving as a fore-runner to flowering, which being not important from the economic aspect as contributory to the height of the millable cane, thus tends to create an erroneous conception about the effect of the treatment. This rapid elongation starts much earlier than the emergence of the flag leaf and as it depends on the stage of the growth of the cane even in the same plot, the figures of the measurement of the total height at this period show a great deal of variation between plant to plant requiring a very large number of samples to get an accurate estimate. A modified method to measure the height of the millable cane on the standing crop itself without disturbing its leaves is therefore introduced. It has been observed that when once the internode attains its maximum development, the leaf sheath which till now swathes it tightly shows a distinct tendency to separate from it. This can be readily judged by gently giving a pull to the leaf blade by holding it near the collar mark. The height of the millable cane thus refers to the height up to the transverse mark of the topmost leaf sheath which can be easily separated from the cane by gently pulling the leaf blade. Its comparison with that of the harvested millable cane showed close approximation within five per cent. The method with the complete data will be described in detail in a separate publication.

(*3*) *Circumference.*—This is measured with a cloth tape at the centre of the middle internode of the millable cane. The alternative method of measuring the diameter of the cane by callipers is not followed as, the cane being not perfectly cylindrical, it was found essential to take at least two measurements at right angles to each other at some specific point and when once the cane is lodged this was found to be very difficult and time-consuming. The measurement of the circumference with the cloth tape which can be passed round the internode very easily without the necessity of handling the cane is found to give as accurate results without the disadvantages of the calliper method mentioned above and is therefore adopted throughout.

(*4*) *Leaf area.*—As the determination of the exact leaf area is rather a laborious process, this is represented by the length and width of the leaf.

Only fresh, fully exposed leaf is taken for measurement per each plant as it is considered to give a true estimate of the effect of the treatment. The length is measured from the collar or throat mark to the tip of the leaf blade, while the width is taken at the centre of this total length.

Except as otherwise stated, thirty-six plants per treatment selected at random were taken for all these botanical observations.

Chemical

(*1*) *Glucose.*—This is estimated in the juice by the methylene blue method of Lane and Eynon [1923].

(*2*) *Amide nitrogen.*—The method of Vickery and Pucher [1931] of preliminary boiling the juice with sulphuric acid is followed. It is then neutralised with sodium carbonate and distilled with magnesia as usual.

IV. Experimental

A. Relation of germination to the climatic factors

In the Deccan-canal tract, the plant cane is normally planted from mid-January to mid-March while 'Adsali' (18 months' crop) is planted from mid-June to mid-August. With the introduction of the new promising Coimbatore varieties, the method of early planting—mid-October onwards—is also coming into vogue. A great deal of variation has been observed both in the rapidity of germination as well as in the final percentage germination in these different plantings. Apparently this seems to be due to the differences in the temperatures during this period and it is felt therefore essential to find out the optimum temperatures so as to adjust the planting time accordingly.

This problem of the influence of climate is, however, complicated by the fact that sugarcane is propagated by setts which are found to differ greatly in the chemical composition according to their period of growth, previous treatment as well as the season prevalent at the time of planting. Under such circumstances the most conclusive experimental technique would have been to run these experiments all at one time with the same planting material but with varying treatments of controlled temperatures. This, however, involved large outlay and was not found to be practicable. Attempts have been therefore made to elucidate this problem by field experiments supplemented by laboratory investigations. For this purpose special plantings were done in six replicated rows at different periods from mid-November to end of February during the last two years and quantitative data on periodical germination and atmospheric as well as soil temperatures (four days counts) were collected. In one series the planting material was obtained from the same treatment throughout, while in another the effect of different manurial treatments was also tested. In all cases, however, the planting material was analysed for amide nitrogen and glucose—the important food constituents in the metabolism of the germinating bud. The results of only one of the series are given in Table I, with only three weeks germination and average atmospheric temperatures during this period.

TABLE I

Relation of temperatures to germination

(Average of six replicates with 90 eye-buds in each)

Serial No.	Date of planting	Germination at 3 weeks		Average temperatures of 3 weeks			No. of days with minimum temperature below 50°F. within the period	Amide N (mg. per 100 grms. of juice)		Glucose (grms. per 100 grms. of juice)	
		Pundia (per cent)	P O J 2878 (per cent)	Max. (°F.)	Min. (°F.)	Mean (°F.)		*Pundia*	P O J 2878	*Pundia*	P O J 2878
1	2	3	4	5	6	7	8	9	10	11	12
1	26th November 1936 . .	18·7±1·03	3·7±0·67	83·6	51·5	65·5	5	0·78	1·47	3·65	1·22
2	10th December 1936 . .	3·1±0·58	2·4±0·77	84·6	49·3	66·9	13	1·05	2·13	2·40	0·78
3	26th December 1936 . .	3·5±1·62	1·7±1·23	83·5	42·8	63·1	22	1·47*	2·01	2·08	0·56
4	9th January 1937 . .	6·3±0·54	14·8±1·65	86·8	47·9	67·3	14	1·89	2·25	1·82	0·30
5	26th January 1937 . .	39·5±3·17	51·3±1·40	89·1	54·7	71·9	4	1·61	2·52*	1·71	0·24
6	10th February 1937 . .	23·7±2·08	28·7±1·97	89·6	53·8	71·7	4	1·56	2·80	1·62	0·21
7	26th February 1937 . .	39·3±3·55	48·7±3·42	95·4	57·8	76·6	1	1·27	2·38	1·53	0·55

* These figures have been obtained by interpolation.

TABLE II

Relation of temperatures to germination

(Average of 12 replicated rows with 105 eye-buds in each)

Period of observations	1934 (Date of planting 16-1-1934)				1935 (Date of planting 18-1-1935)			
	Germination per cent within the period		Average atmospheric temperature within the period		Germination per cent within the period		Average atmospheric temperature within the period	
	Pundia	P O J 2878	Maximum (°F.)	Minimum (°F.)	*Pundia*	P O J 2878	Maximum (°F.)	Minimum (°F.)
1	2	3	4	5	6	7	8	9
23 days from planting	9·4	1·5	85·5	48·1	30·9	24·4	89·5	52·0
24 to 31 days from planting	19·7	14·1	92·8	52·2	16·3	32·5	90·8	53·0
32 to 39 days from planting	16·4	22·0	93·8	54·9	11·8	13·5	92·4	50·6
40 to 47 days from planting	7·6	13·2	94·5	53·7	6·2	9·2	90·8	51·7
48 to 55 days from planting	3·6	6·5	92·5	54·9	5·0	1·8	92·7	51·6
Total germination per cent (8 weeks) . .	56·7	57·3	...	...	70·2	81·4	...	...
Total germination per cent (10 weeks) . .	61·2	64·5	...	...	...	...	...	...

As the germination generally takes from 6 to 8 weeks for its completion, the figures for three weeks germination are expected to give a very good idea about rapidity which, as would be shown later, also influences the total germination. The comparison of these figures with the chemical constituents brings out clearly the influence of the climate. While with the advanced age of the planting material, there is a tendency towards the fall in the assimilable food reserves especially glucose (cols. 8 to 11), there is a significant increase in the germination percentage which can be attributed only to the rise in the minimum temperatures. The inhibitory effect of the minimum temperatures below 50°F. is also evident.

In order to further differentiate between the relative importance of the temperatures and chemical constituents, these data have been put to the statistical test of correlation. It would be seen that there is a high correlation with the minimum temperatures ($+0{\cdot}72 \pm 0{\cdot}037$) while none has been obtained with amide N ($+0{\cdot}31 \pm 0{\cdot}067$) or with glucose ($+0{\cdot}03 \pm 0{\cdot}074$).

It must, however, be emphasised here that this lack of correlation should not be interpreted as non-influence of the chemical constituents on germination. It only clearly brings out the predominating influence of the temperatures. As would be shown later, there is a distinct variability in the germination according to the previous treatment of the planting material even when planted under the same conditions of temperature, and under these circumstances the influence of the chemical nature of the material would be quite apparent.

The advantages of the speed of germination are two-fold. It leads to the increase in the total germination. This is very well illustrated in Table II, which gives the comparative results of germination in a large-scale experiment for two years, one of which has experienced low temperatures.

The material for planting used in both the years had received exactly the same previous treatment and was of the same age, the planting time being mid-January in both the cases. No analysis of the material was carried out, but under these circumstances there would be no great variation in its chemical composition. The slow rate of germination in the first three weeks during the season of 1934 is therefore due to the low temperatures prevalent during the period. During this season, some of the buds sprouted even after eight weeks—the normal period required for complete germination. The total germination even after ten weeks has, however, been much less. The influence of the rapidity on the final success in germination is also evident from Figs. 1 and 2.

FIG. 1. Green *vs.* dry-sealed buds (periodical germination)

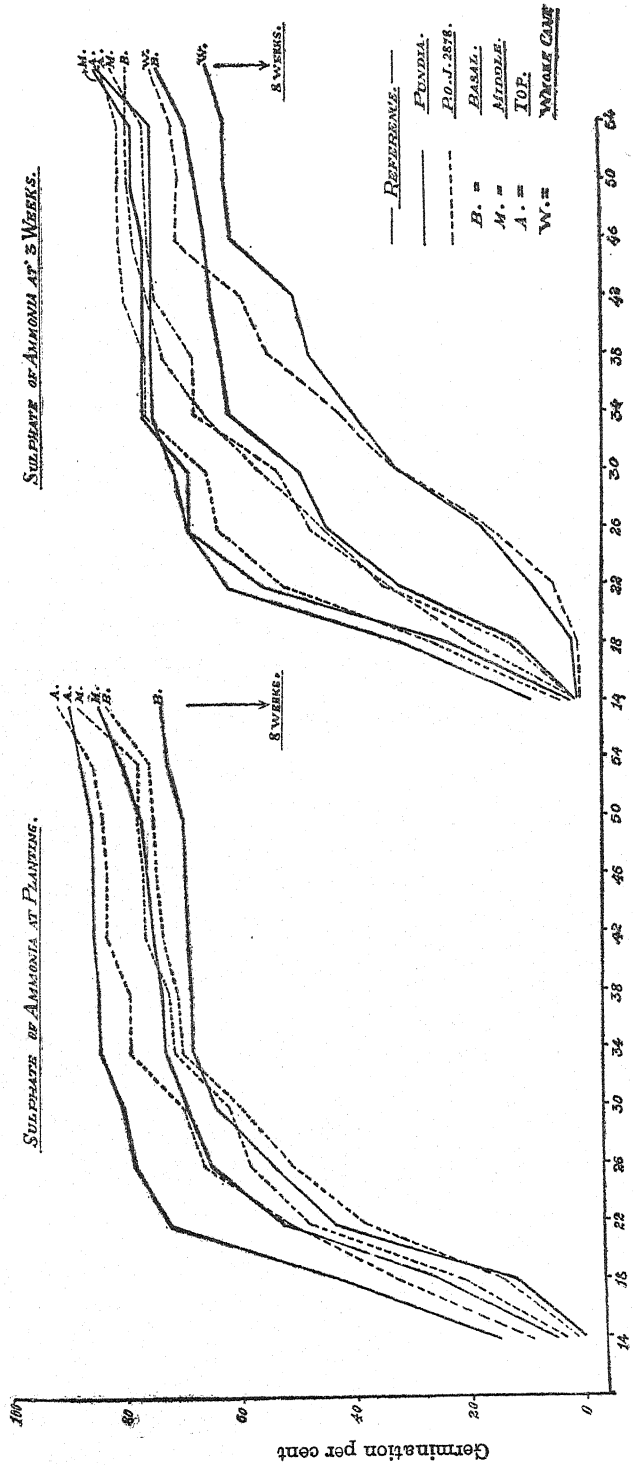

FIG. 2. Periodical germination of basal, middle and top setts

The inspection of the crop has shown that shoots of early germinated buds are generally vigorous and are thus capable of withstanding the borer attack to a great extent. Attempts were further made during the season of 1935 to find out whether this vigour is continued throughout the growing period. For this purpose it was proposed to label ninety shoots from each of those germinated within three, six and eight weeks from two varieties and record their periodical growth by botanical observations. In the case of eight weeks, only about half the number of plants was available at start due to quick germination during this season and this was further greatly reduced by the borer attack, so that by August the observations in this case had to be dropped due to the availability of very few number of plants. The results are given in Table III.

TABLE III

Developmental studies

(Average of 90 labelled plants; date of planting 19-1-35)

	2 months total height	6 months total height	10 months		Harvest time	
			Height of millable canes	Circum-ference	Height of millable canes	Circum-ference
1	2	3	4	5	6	7
	(In.)	(In.)	(In.)	(In.)	(In.)	(In.)
Pundia—						
3 weeks plants . .	12·7	20·0	62·9	4·3	88·7	4·6
6 weeks plants . .	11·0	19·1	59·6	4·3	83·4	4·4
POJ 2878—						
3 weeks plants . .	12·9	26·7	72·6	3·9	94·4	3·9
6 weeks plants . .	12·0	25·8	72·2	3·7	95·1	3·9

It would be seen that while there are indications of a better performance during early stages in the case of plants germinating within three weeks, this advantage is not maintained throughout the growth period and as a result no significant differences are seen at harvest time between these and the plants germinating within six weeks. In the January planting, further delay in germination is, however, definitely deleterious specially due to intense borer attack.

B. Soil—its influence on germination

The soils of the Deccan-canal tract can be included in the broad group of regur or black cotton soil. This is at present being surveyed according to the modern method of genetic soil classification by the Soil Physicist of the scheme, who has so far identified some eight distinct soil types varying in physical and chemical characteristics. It is felt that the system of planting in vogue may not be suitable to all the types and would have to be varied according to their physical nature.

The general system of planting prevalent in this tract is to open out furrows, irrigate them and after allowing the water to soak for some time, press the setts with foot keeping the eye-buds at the sides. Further, short

period irrigations called '*ambavni*' and '*chimbavni*' are given at intervals of four to five days after which regular irrigation at ten days interval starts. There is thus no dearth of moisture to the sett throughout its germinative period. One of the essentials for successful germination is found to be, however, not to plant them too deep in the soil. Planting to a depth of one to two inches is found to be most suitable in all the seasons while during favourable temperatures, surface planting, *i.e.*, planting in flush with the surface of the soil leads to a greater rapidity in germination. In the case of the occurrence of low temperatures, however, there is the risk of the damage to the tender eye-buds due to shrivelling in the latter method.

During the first year (1933) of our experiment in the heavy soil, it was found that in this soil this method of planting leads to the placing of the setts at a much lower depth than is essential for good germination. The very low germination (50 per cent) obtained during this year in spite of favourable temperatures was actually traced to this fact by digging out the ungerminated setts and measuring the depth at which they were lying. It has been observed that after heavy irrigation or rain, such soils get puddled and soft and in walking over them the foot sinks easily into the soil. There is thus always the risk of deep planting by this method which would require a very careful manipulation in pressing the setts. Pressing the setts down into the soil by the hand instead of the usual method of pressing down by foot leads to some improvement; but the most suitable system is found to be the dry planting method, in which case the setts are placed in position in the furrows with just sufficient dry soil to cover them. The furrows are then irrigated with a very slow flow of water in order to minimise the accumulation of soil on the surface. Such slow irrigations will have to be continued for the first three or four irrigations till the soil at the sides of the furrows settles down, after which the system of normal irrigation may be followed. It has given 60 per cent success during unfavourable temperatures of 1934, while under normal conditions about 70 to 80 per cent germination has been obtained. The method also enables efficient supervision on the workmen, as the whole process is capable of inspection without any damage to the sett, and in case of its deeper placing, the mistake can be immediately rectified before irrigation. The Imperial Sugarcane Breeding Station at Coimbatore has now evolved some varieties capable of thriving in soils having bad tilth (chopanised soil). The main difficulty is, however, to get the eye-buds to germinate in these soils. With the adoption of this method of planting, however, the Agricultural Section has been able to secure a very good germination with these varieties.

It has been also observed that in the usual method of planting there is a possibility of deeper embedding of the setts in other soils as well, mainly due to carelessness or ignorance on the part of the workmen. In such cases, however, skipping off one or two irrigations has resulted in great improvement. In fact, it has been our experience that the reduction in the number of irrigational turns helps in accelerating the rate of germination and the second short period irrigation (*chimbavni*) is entirely superfluous, while even the first (*ambavni*) should be dropped in case there is not much cracking of the soil after initial soaking.

C. Eye-buds—influence of their morphological characteristics on germination

According to Earle [1928], morphological bud characters are less variable and less dependent on growth conditions than any other part of the cane plant and can be mainly depended upon for recognising the varieties. It was therefore felt that the variability in the rate of germination among the varieties even under the optimum environmental condition of soil and climate described above might be due to the inherent differences in these bud characters of the varieties themselves. A careful examination of the buds was, therefore, undertaken as regards their size, shape and the position of the point of germination in relation to their germinative capacity. The results, however, did not show any correlation with the differences in these characters. Even in the same cane, the variability observed in the size and shape of the eye-buds at different positions has not been found to influence their power of germination in any way. It was, however, found that the rapidity of germination in the different varieties is greatly affected by one important factor, *viz.*, the presence of dry scale leaves on the buds. The proportion of such buds vary considerably from variety to variety. Further they are found all along the cane although their number is greater in the bottom portion which is known to be very slow in germination. The cause of the drying of the scale leaves of the eye-buds is not exactly known. This process of drying progresses from outwards and buds with a varying number of dry scales are met with on the same cane. In extreme cases only, when the drying extends to the growing point, the bud sometimes presents a shrivelled appearance or becomes soft to the touch with the complete cessation of its activity. During the selection of planting material, such eye-buds are invariably eliminated. In other cases, in which the drying is only in the superficial leaves, the buds otherwise retain their healthy appearance and are quite capable of germination, and the general practice in this tract is to include them in the planting material. In future discussions such buds are termed, for the sake of convenience, dry-scaled buds, while buds with all the scales green are called green-scaled ones.

The observation about the retardation in the rate of germination due to the drying of the scale leaves is quantitatively tested by two types of field experiments. In the first one the germinative capacity of dry-scaled buds and its influence on the development of the crop and yield have been compared with those of the green ones. For this purpose, these two types of buds were separated from the whole cane—about twelve months old—and were planted in different rows. Their periodical germination is illustrated in Fig. 1. A definite decline in the rate of germination is evident in the case of dry-scaled eye-buds which is also reflected in their total germination. In the case of the green-scaled buds the columns bring out an interesting fact that with the application of sulphate of ammonia at the time of planting, the figures for the total germination are almost similar and thus by this method of selection and treatment, the variation in the germinative capacity between the varieties will be greatly minimised. The data about the development of the crop and cane yields are given in Table IV.

TABLE IV

Green-scaled vs. *dry-scaled buds*

(Average of two replicated rows, each row containing 90 single-budded setts)

Variety and buds planted	Sulphate of ammonia at start							Sulphate of ammonia at 3 weeks						
	Per cent decrease in germination at 8 weeks of dry-scaled buds over green	Ratio of tillers to mother cane at harvest time (per cent)	October		Per 3 ft. strip at harvest time			Per cent decrease in germination at 8 weeks of dry-scaled buds over green	Ratio of tillers to mother cane at harvest time (per cent)	October		Per 3 ft. strip at harvest time		
			Height of millable canes (in.)	Circumference (in.)	No. of canes	Weight of canes (lbs.)	Decrease in cane weight of dry-scaled buds over green (per cent)			Height of millable canes (in.)	Circumference (in.)	No. of canes	Weight of canes (lbs.)	Decrease in cane weight of dry-scaled buds over green (per cent)
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
E K 28—														
Green .	—24·3	—1·4	69·3	4·3	6·9	31·0	—28·1	—24·6	—26·2	74·2	4·9	4·5	22·2	—34·2
Dry . .		—7·5	68·9	4·1	4·9	22·3			—28·4	60·8	4·4	3·3	14·6	
POJ 2878—														
Green .	—39·0	48·1	80·2	3·5	11·4	32·0	—18·8	—26·4	54·4	55·3	3·7	8·8	25·9	—21·2
Dry . .		87·2	61·2	3·4	8·8	26·0			81·0	41·8	3·7	7·6	20·4	
Co 360—														
Green .	—28·9	36·8	72·3	3·3	10·4	29·9	—1·0	—26·3	—2·6	59·9	3·6	7·4	21·5	+5·1
Dry . .		111·1	62·3	3·0	11·4	29·6			71·4	68·5	4·0	9·6	22·6	
Pundia—														
Green .	—38·7	—2·7	69·0	4·5	7·3	24·0	—26·1	—66·2	—61·5	...	...	2·5	...	...
Dry . .		0·0	63·7	4·4	4·6	17·6			36·4	...	...	3·0	...	

Except in Co 360, the falling off in germination in the case of dry-scaled buds has influenced the yields, although due to greater tillering (columns 3 and 10) as a result of more available space, the deleterious effect of the low germination is reduced to some extent.

In the other experiment, the dry-scaled buds were entirely left out of the planting material. The experiment was conducted with two varieties—*Pundia* and P O J 2878—and the method consisted of dividing the individual canes (of the same age as used in the previous experiment) in three equal portions from each of which three-budded setts possessing green scales only were selected as planting material. The germination counts are taken every four days after the first fortnight from planting and these are plotted in Fig. 2. There is a distinct fall in the rapidity of germination of the buds from both bottom and middle portions when compared to that of the buds in the top portion. By the time of the completion of the germination however, a close agreement is seen between the curves for middle and top portions while that for the bottom one shows slightly lower trend. This difference between the germinative capacity of the three portions of the cane does no doubt indicate that there are other influencing factors besides the morphological characteristics of the buds; but when the curves for these different portions are compared with those for the general method of planting (plotted in addition under "sulphate of ammonia at three weeks" from the figures of the adjoining plot) in which three-budded setts from the whole cane are utilised for planting without the elimination of dry-scaled buds, significant improvement in the rapidity of germination is evidenced by this method of selection even in the basal portion. Further development of the crop and yield data have also shown that this slight decrease in germination in the basal portion is apparently obliterated during the period of growth (Table V) and it is thus quite evident that by this method of selection of eye-buds, good germination and yield can be secured, whatever may be their position on the cane itself.

TABLE V

Periodical germination and harvest time data of bottom, middle and top setts (Averages of 3 replicated rows with 35 three-budded setts per each row)

Treatment	Germination per cent			Harvest time data				
	Three weeks	Six weeks	Eight weeks	Height of millable cane	Circum-ference	No. of canes per 3 ft.	Weight per cane	Brix
1	2	3	4	5	6	7	8	9
				(in.)	(in.)		(lbs.)	
P O J 2878—								
Sulphate of ammonia at start—								
Bottom	27·3	73·0	82·8	96·4	3·8	8·2	3·2	21·47
Middle	44·4	76·0	86·9	98·8	3·9	8·0	3·0	21·72
Top	48·2	83·0	91·4	99·7	3·9	8·7	2·8	21·69
Sulphate of ammonia at 3 weeks—								
Bottom	29·8	75·0	78·1	94·1	3·7	7·8	3·3	21·72
Middle	29·6	74·0	81·2	94·4	3·95	8·0	3·2	21·22
Top	41·6	79·0	82·5	88·2	3·9	8·9	3·3	20·72
Pundia—								
Sulphate of ammonia at start—								
Bottom	36·2	68·5	73·0	88·7	4·7	5·3	4·0	17·70
Middle	42·5	47·5	84·4	84·4	4·2	7·6	3·8	17·97
Top	64·1	85·0	88·9	90·5	4·3	5·1	4·0	17·70
Sulphate of ammonia at 3 weeks—								
Bottom	26·6	64·0	73·0	86·0	4·3	4·4	4·3	18·22
Middle	46·3	75·5	83·8	78·3	4·6	6·5	3·7	18·15
Top	47·6	74·0	83·2	86·8	4·7	6·4	4·2	18·40

In order to find out the ratios of dry-scaled buds to green-scaled ones in the different varieties, a number of canes twelve months old, under the same treatment were examined for a few varieties. For this purpose individual canes were divided into three equal portions, on each of which separate counts were taken. The results are given in Table VI and are the mean values of 50 randomly selected canes.

TABLE VI

Per cent ratio of green and dry-scaled buds

Percentages are calculated from the mean values of 50 randomly selected canes

Variety	Bottom one-third		Middle one-third		Top one-third		Total	
	Green	Dry	Green	Dry	Green	Dry	Green	Dry
1	2	3	4	5	6	7	8	9
Co 419	57·9	42·3	85·5	14·5	95·9	4·1	79·8	20·3
Pundia	48·5	51·5	84·2	15·8	99·2	0·8	77·3	22·7
Co 360	72·6	27·4	88·3	11·7	96·0	4·0	85·6	14·4
H M 320	16·0	84·0	45·5	54·5	77·3	22·7	46·3	53·7
P O J 2878	39·0	61·0	84·4	15·6	97·9	2·1	78·8	26·2
E K 28	8·7	91·3	36·3	63·7	63·3	36·7	36·1	63·9

It would be seen that the basal portion contains the largest number of dry-scaled buds and the top portion the least in all the varieties. Among the varieties, E K 28 possesses the largest number of dry-scaled buds and this variety is known to give very low germination. The experimental evidence about the deleterious effect of dry-scaled buds adduced above thus conclusively demonstrates that a great deal of variability in the germinative capacity among the varieties can be reduced by the elimination of dry-scaled buds as the different proportions of these buds seem to be the main cause for this variation. Further with the application of sulphate of ammonia at the time of planting to green-scaled buds uniform germination is secured (Fig. 1) in all the varieties tested. This information about the selection of the eye-buds would therefore be very important from the standpoint of the practical agriculturist, who, because of the variable germination that he is at present getting from the different varieties is perplexed as to the number of setts he should plant.

It has already been stated that except for a few dry scale leaves, the buds otherwise exhibit outwardly quite a healthy appearance and are capable of germination. What are then the physiological changes which bring about this retardation in the rate of germination ? One of the effects of the presence of the dry scale leaves is found to be the reduction in the water content of the buds. Dry-scaled eye-buds from twelve months old crop under the same treatment were analysed for their moisture content in comparison with the green-scaled ones. Table VII gives the average figure of four independent determinations in each variety.

TABLE VII

Moisture content of green and dry scaled eye-buds

(Average of four independent determinations on ten buds per each determination)

Variety	Eye-buds	Percentage moisture*		
		Original	After soaking for 24 hours	Absorption
1	2	3	4	5
1 P O J 2878 . .	Green . .	69·5±0·68	80·7	11·2
	Dry . .	60·3±2·11	76·2	15·9
2 *Pundia* . . .	Green . .	71·3±1·34	79·1	7·8
	Dry . .	66·0±0·99	76·0	10·0
3 Co 419 . . .	Green . .	81·9±0·95	82·7	0·8
	Dry . .	75·8±1·30	78·1	2·4
4 Co 413 . . .	Green . .	81·1±1·31	83·1	2·0
	Dry . .	76·1±2·12	78·8	2·7
5 Co 360 . . .	Green . .	84·8±1·47	85·0	0·2
	Dry . .	76·1±0·95	77·9	1·8

* While the determinations of the moisture content of both types of buds per variety were made at the same time the varieties were taken at two periods due to the non-availability of all the varieties at the same period. Thus the determinations for the varieties 1 and 2 were done in March, while those for the rest were done in Septemper 1937. The h'gher moisture content in the latter is due to this.

It will be seen that in all the varieties there is a definite lowering of the moisture content in the dry-scaled buds in comparison with that of the green-scaled ones. Further studies on the absorption of moisture after soaking for twenty-four hours have shown that the dry-scaled buds are capable of absorbing water rapidly (Table VII, columns 4 and 5). Interesting differences between the two types of buds are, however, found from their microscopic examination. In the green-scaled ones, there is a slight swelling of the scales after soaking thus facilitating their easy separation. In the other case no change has been observed due to soaking, the dry scales remaining sticking to the inner scale leaves as tightly as in their original condition. Bonazii [1928] has maintained that the germinability of cane buds is directly correlated with water and glucose content of the meristematic tissues of the eye-buds. As will be shown later, no correlation of glucose with the germinative capacity of the buds has been observed by us under the conditions of our experiment. As the dry-scaled buds are found to be capable of absorbing

water very rapidly, this should not be also a limiting factor under the conditions of irrigation. We are therefore inclined to the view that the delay in germination in the case of dry-scaled buds is either mainly due to the mechanical obstruction to the emergence of the growing point or to the retardation of gases to or from it. The removal of dry-scale leaves from such buds by careful teasing with needles has in fact resulted in the acceleration in the rate of germination (Table VIII).

TABLE VIII

Effect of the removal of dry-scale leaves of buds on germination

(*Average of 2 replicates with 27 eye-buds in each ; Variety—P O J 2878 ; germination per cent*)

Kinds of buds planted	Days after planting								
	16	20	24	28	32	36	40	44	48
With dry scales .	0	0	0	11·1	13·0	18·5	27·7	27·7	48·1
With dry scales removed	0	1·9	8·1	18·5	31·5	35·2	38·9	38·9	51·9

In the Deccan-canal tract the whole cane is used as planting material and usually these canes are taken from the ordinary plantation without maintaining special nurseries for the same. The ratio of dry-scaled to green-scaled buds given in Table VI shows that in some of the varieties, the proportion of dry-scaled buds is very great and their elimination may not be thus practicable. The improvement in their germinative capacity by the removal of dry-scale leaves suggested a possible solution ; but the method is too laborious requiring careful manipulation and will be thus impracticable. Investigations are in progress to find out a suitable method for the utilisation of such buds and these will be dealt with in a later publication.

D. Type of the sett—its influence on germination

The general practice in this tract is to utilise three-budded setts for planting. It was felt, however, that with the above knowledge about the selection of eye-buds there would be a greater possibility of rigid selection if these were substituted with the single-budded setts as the planting material. Kulkarni [1918] has in fact shown a very high germination in the case of single-budded setts in comparison with the three-budded ones. His method of planting, however, differed in both the cases, the former being planted with the bud upwards, while in the case of the latter, the buds were kept sidewards. Even with the sideward method of planting in both the cases, we have observed a higher rate of germination in the single-budded setts. These figures are represented in Fig. 3. The better performance in the single-budded sett is considered to be mainly due to the possibility of its better selection. The low temperatures experienced during the season of 1934 have affected the germination of this year in the case of *Pundia*. It is observed that under such circumstances single-budded setts are likely to be damaged by these temperatures much more quickly than the three-budded setts.

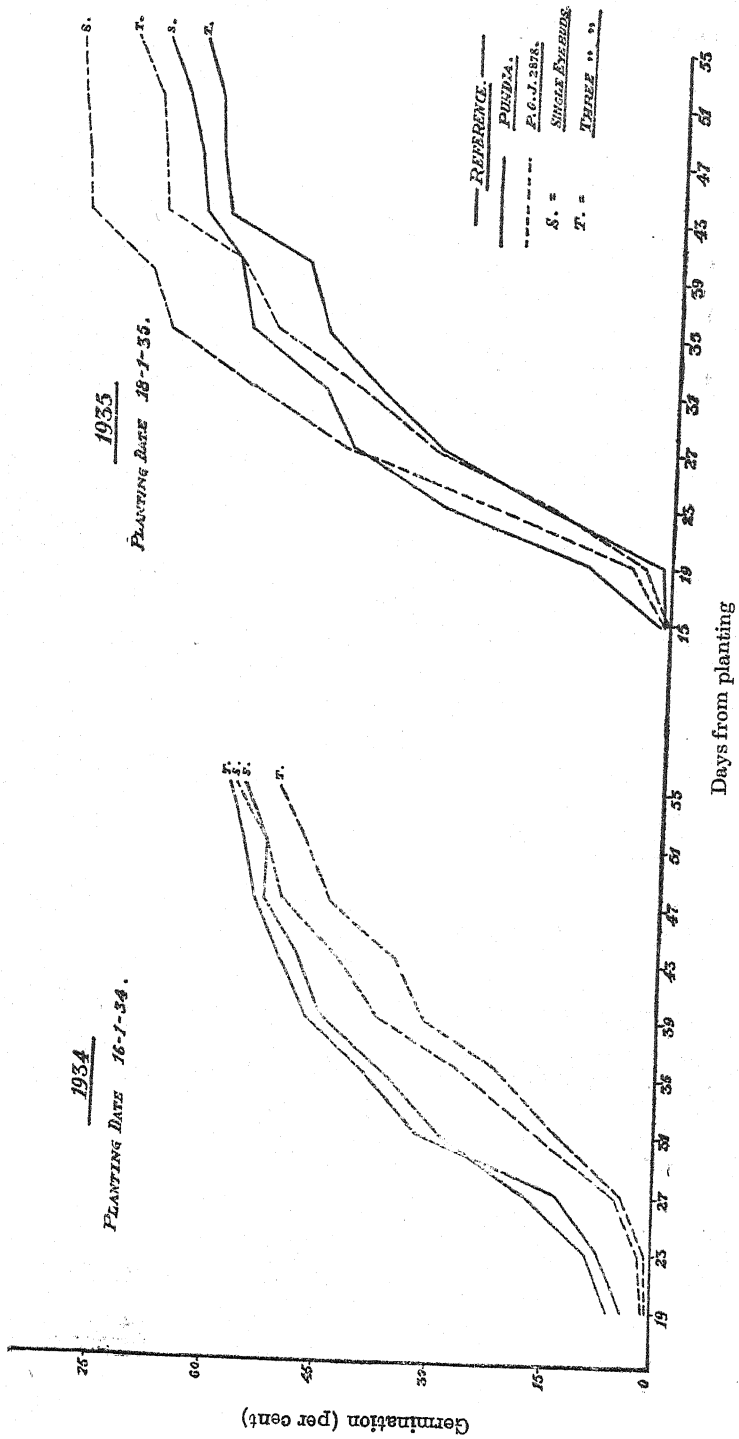

Fig. 3. Single *vs.* three eye-bud setts (periodical germination)

Inspection of the crop in both the series, however, showed the plants from the single eye-bud setts to be not so vigorous as in the case of the three eye-bud setts. These were invariably thinner with less height and the leaves appeared to be narrower than in the other case. This effect was very pronounced during the early stages of growth—for four to five months from planting but was hardly visible at a later stage—about a month after earthing up. A special experiment was therefore laid out to test this quantitatively with one variety—P O J 2878 only—under four manurial doses varying from 225 to 600 lbs. nitrogen. The crop was harvested periodically, seven 3-feet strips being cut at each period and weighed separately. For this purpose, the two end rows and 4-feet on either side of the intermediate rows were excluded from sampling in order to avoid the border effect. The remaining lengths of the rows were then divided into 3-feet strips from which only alternate strips were utilised for sampling by random selection. By this method there was no possibility of any of the selected strips abutting on the previously sampled one. The results of variation in yield are as follows.

TABLE IX

Single eye-buds vs. *three eye-buds*
(*Variety, P O J 2878*)

Treatments	Per cent variation in yield of single eye-buds from three eye-buds (Average of seven 3-feet strips)		
	July	October	Harvest time
225 N	—65·8	—30·0	—16·2
300 N	—68·4	—45·1	—16·0
450 N	—41·7	—18·8	+18·4
600 N	—24·7	—28·2	—14·1

It would be seen that there is a definite lowering of the physiological performance throughout the earlier stages of the life-cycle of the plant in the case of single eye-bud sett. Due to controlled conditions of irrigation and manuring these differences are, however, greatly minimised with the progress of growth specially in the higher nitrogenous treatments. The higher yields reported by Kulkarni [1919] in the case of single-budded setts are due to his adopting the artificial method of removing all the tillers as they appeared and allowing only the mother canes to develop in both the treatments. This prevented the other treatment from adjusting by tillering the fall in the number of plants as a result of less germination.

While in the normally cut single-budded setts, the germinative capacity is not in any way lowered, further reduction in the size of these setts by cutting them close to the eye-buds is found to affect both the speed of germination as well as the total success.

TABLE X

Performance of close-cut and ordinary single-budded setts

(*Average of 112 eye-buds*)

Type of setts planted	Germination (per cent)			At 4 months		
	Three weeks	Six weeks	Eight weeks	Total height (in.)	Length of leaves (in.)	Breadth of leaves (in.)
Close cut—						
Single eye-buds . .	16·2	38·4	42·3	14·5	44·1	2·03
Ordinary—						
Single eye-buds . .	20·4	53·2	53·9	17·4	46·5	2·12

It is thus quite evident from all this experimental evidence that the reserve material in the seed piece not only makes a definite contribution to the nutrition of the developing plant in the early stages of its growth but even exercises an important control on the process of germination.

E. Chemical basis of germination

It is a matter of general belief that buds from either immature canes or immature portions of canes germinate quicker than those from the mature ones, and on this basis canes eight to ten months old are considered as the suitable planting material. It has, however, been found that due to the susceptibility to the borer attack, the March or April plantings for securing this type of planting material for the January season are not advisable. It would thus be necessary to find out a suitable alternative method to overcome this difficulty. The differences in the germinative capacity of the different portions of the cane, which are very well illustrated in Fig. 1 (already discussed in section C) where in spite of careful selection of buds, the rate of germination in the bottom portion is lower than in the other two portions, suggested the possibility of the chemical composition of the reserve material of the sett as a predominating factor. In order to throw further light on this, three-budded setts from the canes of the same age but under varying nitrogenous treatments were planted in sand, washed free from all soluble constituents, so as to entirely eliminate the effect of soil nutrients. These experiments were repeated at three different periods with the planting material of eight, ten and twelve months of age, the number of eye-buds being 90 per each nitrogenous treatment. As the different plantings have shown similar trend in germination, the results of only the last period in which the planting material was of twelve months age are demonstrated in Fig. 4. It is quite evident from the increased rate of germination in the case of the planting material obtained from the higher nitrogenous treatments that the age of the planting material should not be the only criterion in its selection. From our knowledge of the reserve food materials on the process of germination and the initial growth of the shoot as discussed in section D, this variation in the rate of germination can be assigned to the differences in the chemical composition of the setts themselves. The most important constituents from the standpoint of the process of germination are apparently the carbohydrates and nitrogenous compounds. The carbohydrates are utilised in respiration or in other words for the transformation of potential to kinetic energy for pushing out the roots in the soil, the shoots above ground and carrying out the various activities of the seedlings. Miller [1931] in his

experiments with sun-flower seedlings has noted that one-fifth of the carbohydrates stored in the seed have been consumed for this purpose. Among the carbohydrates, hexoses are apparently the sugars utilised in the process, glucose being the most oxidisable one among them. Nitrogen is considered to be important in the anabolic activities of the growing bud, the lower forms of nitrogen being the most suitable for the purpose. In order, therefore, to find out the relative influence of carbohydrates and nitrogen on the rapidity of germination, the planting material from the series in the sand described above was analysed at start for glucose and amide nitrogen and these were put to the statistical test of correlation with the three weeks germination which was taken as the criterion for its rapidity. The results have given a positive correlation with amide nitrogen ($+0\cdot71\pm0\cdot063$), while no correlation is observed with glucose ($+0\cdot32\pm0\cdot115$). This lack of correlation with glucose would be considered rather surprising as it is against the consensus of opinion, which, due to the better performance of the immature canes as the planting material, generally associates glucose with the germinative capacity of the cane. A quantitative interpretation to this has been given by Evans [1936] who has actually shown a negative correlation ($-0\cdot62\pm0\cdot097$) between the reducing sugars and the number of days required for germination. He, however, has expressed the opinion that this relationship does not mean that the high reducing sugar is the cause of quick germination but should be considered only as a reliable index of the condition of the planting material with regard to its germinative capacity. Further light on this question has been thrown by the investigations of Das and his co-workers [1936] who have shown the association of high water content with high glucose content. In our manurial investigations to be described separately, we have also found similar relationship, and explanation as regards this disagreement in the correlations seems to lie in the variation in the conditions under which these studies have been carried out. All our germination experiments have been conducted under irrigation and therefore moisture is not a controlling factor. On the other hand, Evans has worked under dry conditions and the success of planting under these conditions will be chiefly dependent upon the soil moisture. It will be seen that under such conditions, the water content of the sett itself would count. The association of the increased quantity of water with the higher glucose content should thus be apparently an important factor in the correlation obtained by him. The importance of the water content of the sett is also visible from the improved germination he gets with soaking of setts in water or lime solution which is contrary to our findings. When the sett is soaked, it absorbs water, and therefore does not have to depend on the soil moisture to initiate its germination.

FIG. 4. Periodical germination of canes under varying manurial treatments (age of cane 12 months)

No such relationship has been observed between the amide nitrogen and water content of the cane. There are in fact indications of a tendency towards an increase of this nitrogen in the juice of the cane with the cessation of the vegetative activities (Table I). The high correlation observed in this case thus seems to be due to its direct influence on the rapidity of germination itself. This has received further confirmation by the favourable effect of the application of sulphate of ammonia in the furrows themselves at the time of planting. This results in its absorption through irrigational water and the analysis of the planting material shows an increase in its ammoniacal nitrogen content, which leads to the acceleration of the process of germination. (Table V and Figs. 1 and 2). Repeated field experimentation with sulphate of ammonia at the time of planting under varying conditions for three years has shown that while there has been almost invariably some improvement in the rate of germination, its influence is found to be significant under unfavourable conditions of either the seed material, climate or soil. On the other hand there appears to be no necessity of this treatment in the case of the combined favourable effect of these factors as the proper seed material, the presence of optimum temperatures and the retention of good tilth with the proper depth of planting. It is thus quite evident that the pre-requisite for the success of this treatment is the adverse nature of any one or more of these factors which appears to check the enzymic activities leading to the disintegration of protein nitrogen in assimilable lower forms. Out of these factors, it has been already shown that temperatures are predominant and in case they happen to be favourable during the period of germination, they mask to a great extent the adverse effect of the remaining two factors of the seed material as well as the soil.

V. Summary and conclusions

(1) The rapidity of germination is found to be predominantly influenced by the minimum temperatures which in their optimum condition even mask the effect of the chemical constituents of the planted material. Temperatures below 50°F. are definitely injurious.

(2) The advantages of the speed of germination have been observed to be two-fold. It leads to the increase in the final percentage germination. Secondly the shoots of the early germinated buds are vigorous and thus capable of withstanding the borer attack to a great extent.

(3) In order to get the optimum germination, it would be necessary to adjust the method of planting according to the soil type. While the existing method of pressing the sett by foot in the wet soil is found to be suitable for lighter soils, dry planting with slow irrigations improves the germination greatly in heavier soils.

(4) Buds with dry scales are found to be slow in their germinative capacity and the different proportions of these among the varieties appear to be one of the important reasons for the great deal of variability in the germinative capacity of the varieties. With the elimination of these dry-scaled buds from the planting material, germination in the varieties has been much more uniform.

(5) Although slightly higher rate of germination can be obtained in the case of single-budded setts due to the possibility of better selection, the crop in this case is poorer in the early stages of growth when compared to that from three-budded setts. The differences are, however, greatly minimised with the progress of growth, specially in the higher nitrogenous treatments.

(6) Age of the planting material should not be so much the criterion in the proper selection of setts as the chemical composition. Out of the two important chemical constituents required for germination, *viz.*, carbohydrates, mainly glucose, and soluble nitrogenous compounds, the latter has been found to be more important and has actually given a positive correlation, while no such relation has been found with the former. This lack of correlation with glucose seems to be due to our system of planting under irrigation. As a practical example of the importance of nitrogen in germination may be cited the beneficial effect of the application of sulphate of ammonia at the time of planting which is found to be significant especially under unfavourable conditions of soil, climate or seed material. It is thus evident that special seed areas heavily manured with nitrogenous top dressings and the application of sulphate of ammonia at planting will help in securing optimum germination.

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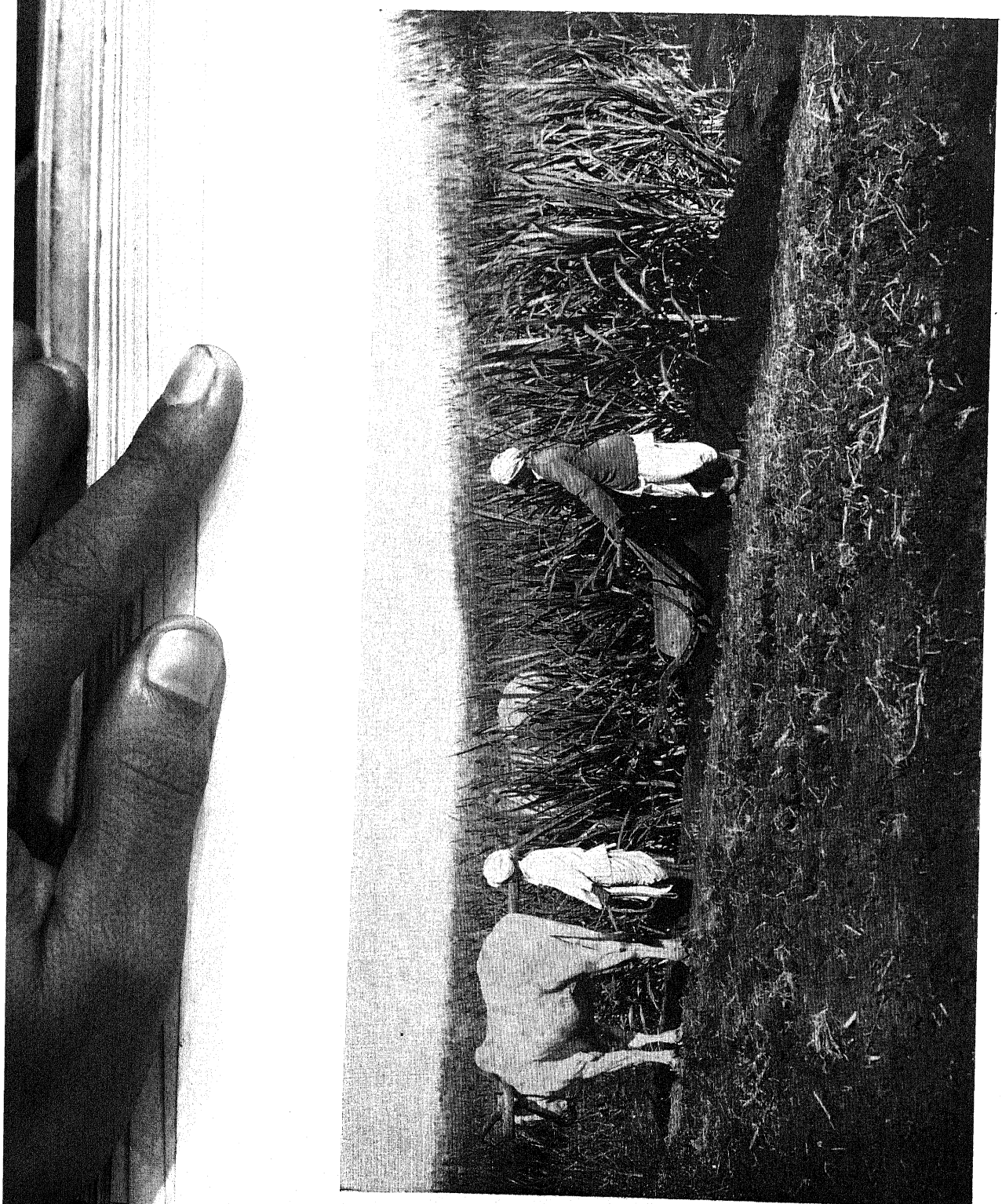
PLATE XIX



Earthing-up of cane with the *Sabul* plough

II. 'EARTHING UP' OF CANE

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(With Plates XIX and XX)

I. Introduction

'Earthing-up' or 'hilling', as it is also called in some countries, is an important operation in the cultivation of sugarcane, not only in India but in several other cane-growing tracts of the world. In the Deccan-canal tract, as also in most other places where sugarcane is grown under irrigation, the setts are planted in furrows, which are usually three and a half to four feet apart. As the cane plant grows and internodes are being formed above the ground level, earthing is done either two or three times or only once after five to six months of planting. In the case of partial earthing, the loose soil on the sides of the ridges is gradually put on either side of the growing plant by manual labour. The final earthing is generally done by the manual labour but can also conveniently be done by Sabul plough fitted with a special tail sheet attached to the mould-board for pressing the loose soil against the row of cane [Bom. Dept. Agric., 1929] (Plate XIX). Before this operation is done, however, all the dry leaves sticking to the canes are removed so as to expose the internodes likely to be covered with soil for facilitating the development of fresh roots from them. A heavy dose of nitrogenous top-dressings is then applied in the furrow near the base of the plant, as it becomes very difficult to manure the crop later. The Sabul plough breaks open the ridges between the furrows which are converted into ridges by getting the earth on either side against the canes, turning at the same time the old ridges into furrows.

The extent of this 'hilling' or earthing-up varies in different tracts. In places like Australia [Venkatraman, 1936] and Cuba [Earle, 1928] the cane crop is not appreciably earthed up so that by the harvest time the crop

* This scheme is partly subsidised by the Imperial Council of Agricultural Research.

stands practically on level ground, thus facilitating the harvesting operations by means of machinery. In the Deccan-canal tract, on the other hand, there is a tendency for a much higher earthing and this operation thus becomes very laborious requiring considerable labour and bullock power. The period of earthing of the major January-planted crop generally coincides with the sowing operations of the dry crops and as the labour is scarce at that time, the operation becomes costly. It is usually given by contract at the rate of Rs. 9 to Rs. 12 per acre. Although the Deccan-canal tract is known for its high tonnage of cane, the cost of production is also very high, being As. 6 to As. 7 per maund. Attempts are therefore directed towards exploring methods which would bring down this high cost and with this object a careful study of the process of earthing up was therefore undertaken.

The main advantage claimed for the process of earthing is its beneficial influence on the growth of cane. It is considered that the cutting off the cane roots during the process of earthing-up induces a fresh flush of feeding roots in the earthed-up zone, and this gives a fillip to the growth of the cane. There is, in fact, a consensus of opinion among sugarcane growers that failure or delay in this operation results in reduced yield. Incidentally, high earthing-up is also considered to give mechanical support to the canes, preventing them from lodging and also, to renew the irrigational system by opening up fresh furrows. The effect of the earthing-up would thus principally be a physiological one and therefore the developmental behaviour of the cane plant with and without earthing-up was carefully followed till harvest time. The results of these observations are embodied in the paper.

II. Experimental

(*a*) *Field experiments.*—These experiments were started on a small scale in 1935-36 with the variety POJ 2878. Of the twelve small-sized plots (0·73 cents each) under observation, six were earthed up and six were not. All the other treatments such as manuring, irrigation, etc., were similar in both cases. The results are given in Table I. It is interesting to note that the results of yields are quite the reverse of the general belief, the tonnage being actually higher in the case of the non-earthed plots, which can be attributed to the larger number of canes in this treatment. In weight per cane and in brix, however, there is a fall which seems to be due to the development of late tillers which would have otherwise been smothered up by the operation of earthing-up.

TABLE I

Earthing vs. *non-earthing*

Planted on 19-1-35, Harvest data (POJ 2878), Harvested on 11-1-36

Treatment	No. of canes per 3 ft.	Average weight per cane	Yield in tons per acre	Brix @17·5° C.
		lbs.		
Earthing	7·5	3·0	36·8	20·24
Non-earthing . . .	9·1	2·7	38·9	19·76

Encouraged by these results the experiment was repeated in replicated trials (plot size 1·74 cents) during the next two years, (1936-37 and 1937-38), there being four replicates of each treatment. The variety was the same, *viz.*, POJ 2878. Records of germination, tillering at earthing time, periodical growth, number and weight of canes at harvest, brix, sucrose and purity were maintained during both the years. The figures are given in Table II.

These figures clearly show that the germination as well as the tillering have been practically the same in both the treatments. In the number of canes at the harvest time, however, there is a distinct increase in the non-earthed treatment during 1936-37 and a slight one in 1937-38. This increase is mainly due to the development of the young shoots which escaped smothering in the non-earthed series as no further tillering was observed in this series after the earthing up of the other one. These tillers are, however, generally late-growing and may some time bring about a slight lowering of the brix or purity. The dissimilarity in the performance of tillers during the two seasons seems to be due to the different rates of germination in them. During the 1937-38 season, the rapidity of germination as indicated by three weeks counts (Table II, col. 1) led to early start in tillering, resulting in a large number of bigger shoots at earthing time, which reduced the loss of tillers by suppression with the process of earthing-up.

TABLE II

Earthing vs. *non-earthing*

(*Variety POJ 2878*)

Year	Treatment	Germination percentage at			No. of shoots at earthing per 3 ft.	No. of canes per 3 ft. at harvest	Percentage success	Weight per cane	Calculated yield in tons	Brix at 17·5°C.	Sucrose (per cent)	Purity (per cent)
		3 weeks	6 weeks	8 weeks								
		1	2	3	4	5	6	7	8	9	10	11
								lbs.				
1936-37 .	(*a*) Earthing . .	14·2	67·4	72·7	14·7	7·9	53·7	2·9	37·2	21·47	19·38	90·2
	(*b*) Non-earthing .	11·5	66·0	73·5	15·3	9·8	64·0	2·8	41·4	21·72	19·78	91·2
1937-38 .	(*a*) Earthing . .	24·7	66·9	70·3	15·0	9·0	60·0	2·9	43·0	21·79	19·63	90·1
	(*b*) Non-earthing .	27·3	68·0	71·0	15·5	9·9	63·9	2·8	44·8	21·79	19·23	88·2

In order to find out whether earthing-up does accelerate the growth to any extent, twenty-five random selected plants in each treatment were labelled at the time of earthing up and the heights of the millable cane recorded at harvest time after topping. The average height agreed very closely in both the cases, being 105·1 inches in the earthed-up and 105·0 inches in the other case. It would thus be seen that the slight fall in the average weight per cane (Table II, col. 7) in the non-earthed treatment is definitely due to the development of late tillers.

The final yield figures were tested for statistical significance (Table III). The experimental layout was a complex one including two other treatments. The data relating to earthing *vs.* non-earthing are alone given.

TABLE III

Yield data in lbs. per plot excluding ring (0·8 cent)
(Variety POJ 2878)

Replicate	1936-37		1937-38	
	Earthing	Non-earthing	Earthing	Non-earthing
I	687	777	752	809
II	739	699	764	858
III	710	813	860	805
IV	622	779	805	842
Mean	689	767	795	828
Critical difference for significance at $P = 0·05$ . .	83		75	

These results show a definite tendency towards increased yields in the non-earthed treatment almost approaching significance in 1936-37, while during 1937-38 the yields are almost similar in both the treatments. It is thus quite evident that absence of earthing does not in any way lead to the reduction in yield.

This experiment was also tried on a field scale. A cane plot 80 cents of POJ 2878 was divided into four blocks of 20 cents each, two of which were earthed-up as usual and the other two were left non-earthed. The yields of the main plots excluding the ring calculated in tons per acre are given in Table IV.

TABLE IV

Yield in tons per acre

(Variety POJ 2878)

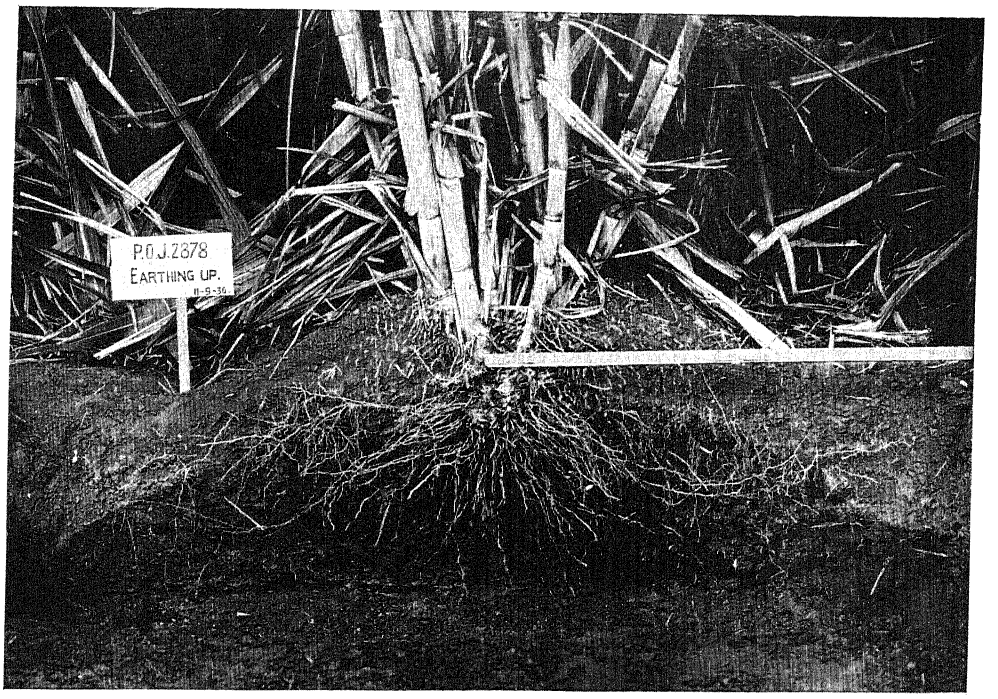
Planted on 9-2-37 Harvested on 6, 7 and 8-2-38

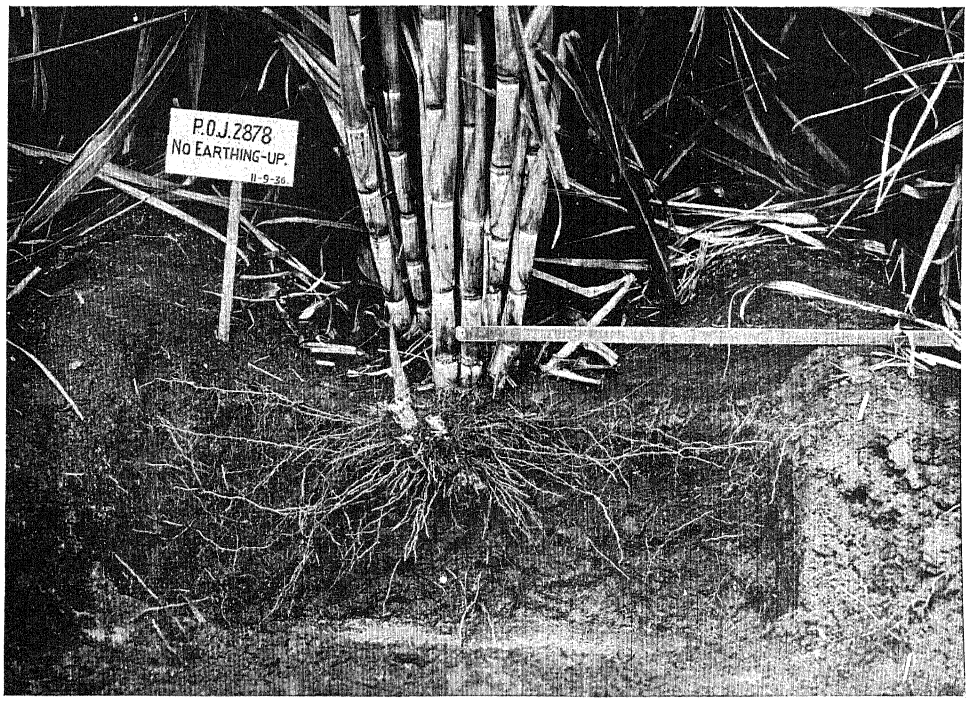
Replicate	Earthing	Non-earthing
I	36·8	40·0
II	39·2	42·4
Mean	38·0	41·2
Brix @17·5°C.	22·5	22·4

There is an increase of 8·4 per cent in the yield of non-earthed crop without any fall in the brix.

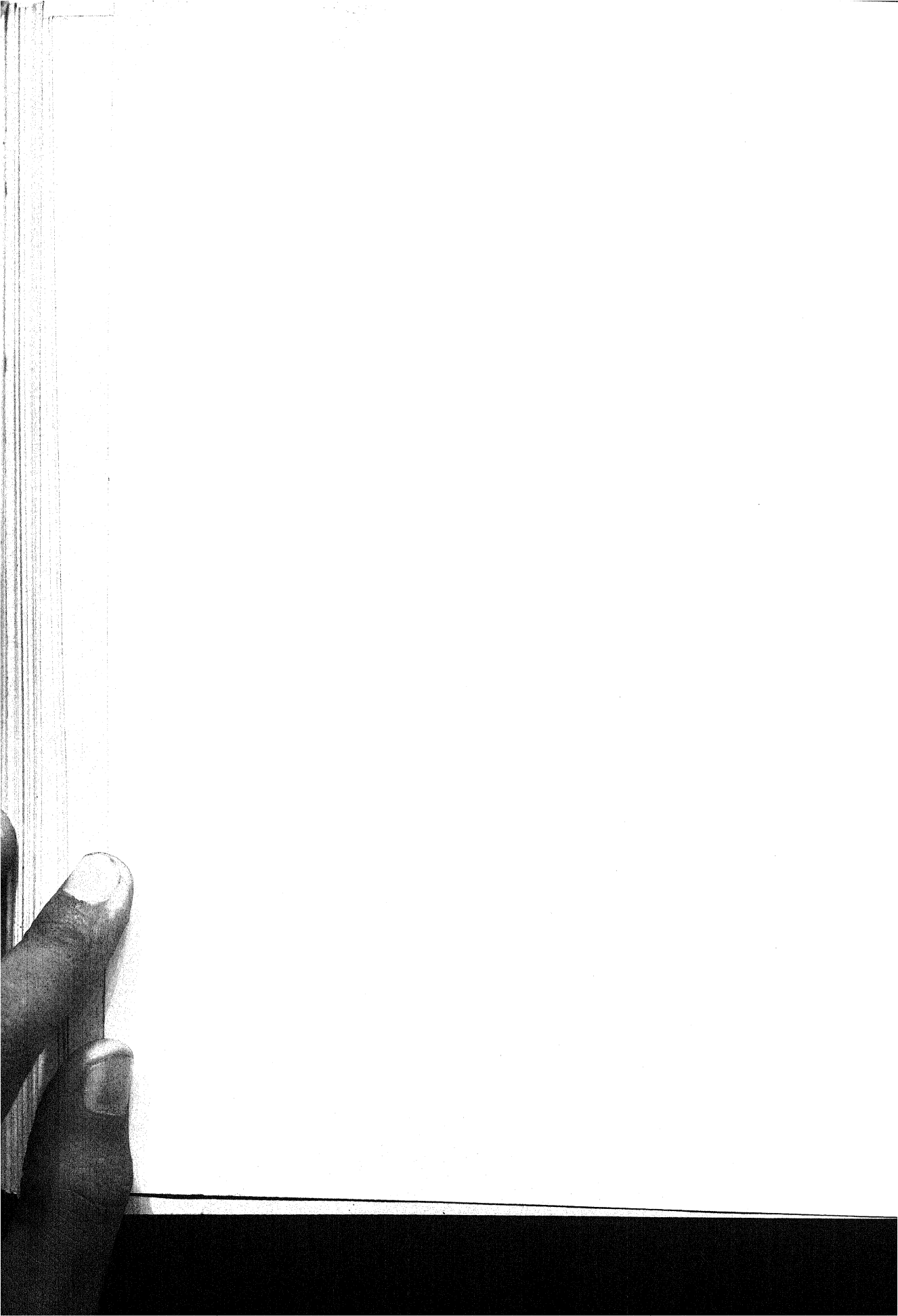
It may be pointed out here that the season (1937-38) was characterised by a heavy downpour of about 8 in. of rain accompanied by a cyclone during September, which caused greater lodging in the non-earthed cane, especially under exposed conditions. In spite of this, there has not been any appreciable fall in the brix or purity in this variety (Tables II and IV).

(*b*) *Root studies.*—With a view to find out if there are any advantages to the root-system by the process of earthing-up, as are usually claimed, careful dissections of the roots of stools were made under both the treatments (Plate XX). It will be seen that there is practically very little difference in the two treatments, except that in the case of the earthed-up stools the fine roots formed after the last dose of nitrogenous top-dressing are found in the earthed-up zone; while in the other case they are restricted to the surface layer of the soil forming a thick network. The difference in the functioning of the roots was studied three and a half months after the operation of earthing





Root system of cane (POJ 2878) with and without earthing



by an analysis of the exudates collected from canes under both the conditions and also after removing the fresh surface roots formed after earthing. The results of these studies are given in Table V.

TABLE V

Exudation studies

($3\frac{1}{2}$ months after earthing-up)

Treatment	Quantity of sap exuded per cane in 24 hours	Absorption per cane per day			
		Nitrates	Ammonia	K_2O	P_2O_5
	(c.c.)	mg.	mg.	mg.	mg.
1. Non-earthing .	63	0·025	0·19	2·63	0·74
2. Earthing . .	36	0·024	0·12	2·55	0·66
3. Earthing with roots in the earthed zone removed . .	40	0·021	0·064	2·83	0·50

These figures clearly show that the root-system in the case of earthing-up remains mainly as surface feeder, while in the absence of earthing it develops, in addition, a capacity to tap deeper layers as well. The large quantity of exudates obtained in the non-earthed treatment also clearly indicates that the crop is capable of taking its water requirement from lower layers and would thus require less irrigational water than in the other case. In fact owing to the slow silting of the furrows with irrigation, the non-earthed crop actually receives less water at every irrigational turn than the earthed-up one where new furrows are opened with the operation of earthing-up, thus renewing the system afresh. This reduction in the quantity of water is not found to produce any deleterious effect on crop growth. There is thus an actual saving of water in the non-earthed treatment which is estimated to be between 10 to 15 per cent. The analysis of the exudate further shows all the mineral constituents to be more available in this treatment than in the other one and it is thus quite evident that the functioning of the root-system is not in any way improved by the operation of earthing up.

III. CONCLUSIONS

The prevalent idea among the sugarcane growers about the necessity of earthing in order to accelerate growth for the improvement in yield is found to be quite untenable on actual experimentation. On the contrary, the treatment which was not earthed has shown a definite tendency towards

higher yields. This can be mainly ascribed to the presence of larger number of millable canes at harvest time in this treatment owing to the development of late tillers which are otherwise smothered by the operation of earthing. Root investigations have also shown that, with the operation of earthing, the root-system is actually impaired as it remains mainly as a surface feeder, while in the non-earthed treatment, it develops a capacity to tap deeper layers as well. It is thus quite evident that the general belief about the beneficial effect of earthing on growth is based on a misconception as the impetus to growth observed after this operation appears to be entirely due to the nitrogenous top-dressing of the crop which immediately precedes it.

The high earthing as practised in the canal tract does, no doubt, act as a mechanical support, especially to the lodging varieties. It is not, however, very important for the erect types which now occupy a major area in this tract. It is only in the case of cyclonic weather that lodging appears in these varieties, specially under exposed conditions and this is observed to be more in the case of the non-earthed treatment. This weather is, however, generally met with in late September or October and lodging at this period is not found to adversely affect the yield or the brix. Another advantage of irrigational facilities claimed for this operation is found to be actually a disadvantage, as the opening of fresh deep furrows leads to the application of higher delta of water than the crop requirement, resulting in the wastage of canal water which is brought to this tract at a very high cost to the Government. It has been observed that in the case of four-feet spacing between furrows at the time of planting as recommended in the standard Manjri method no difficulty in watering is ever experienced till harvest time, the furrows remaining sufficiently deep to accommodate the water required by the crop.

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THE EXCHANGE OF MOISTURE BETWEEN PLANT MATERIALS AND THE ATMOSPHERE

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(With four text-figures)

I. INTRODUCTION

RAMDAS and Katti [1934; 1935; 1936] have discussed, in a series of papers, the phenomenon of the diurnal exchange of moisture during the clear season between a dry soil surface when it contains only hygroscopic moisture and the air layers near the ground. They have shown that the soil has its maximum moisture content at the minimum temperature epoch and its minimum moisture content at the maximum temperature epoch. From the morning after sunrise up to the afternoon, the soil surface gives up moisture by evaporation to the air layers above, but thereafter, until the next morning, it desiccates the air layers by re-absorbing almost all the moisture lost. It was also shown that the intensity of this moisture exchange is greatest in the black cotton soil of the Deccan and least in the alluvial soil of the Indo-Gangetic plains.

Much work has been done by various workers* on the hygroscopic properties of textiles like cotton, silk, wool, etc. Mention may be made of the investigations of Wilson and Fuwa [1922] on the absorption of moisture by a variety of materials including some plant materials like tobacco, wheat bread, flour, etc., when exposed to air of different relative humidities. Nazir Ahmad [1933] studied the seasonal variation of the moisture content of bales of cotton stored in godowns. He showed that cotton absorbed moisture during the monsoon season but lost it again during the dry season. Diatlova [1935] found that there is a diurnal variation in the moisture content of grain and straw of spring wheat ready to be harvested, the highest moisture content occurring at 5 A.M. and the lowest moisture content between 1 and 4 P.M.

During the clear season of 1937-38, experiments, similar to those in the case of the soils, were made on the diurnal variation in the moisture content of various plant materials, at the Central Agricultural Meteorological Observatory at Poona. The clear season is characterised by a large diurnal range of

*For references to literature on this subject see Chapter XII of " The absorption of gases and vapours by solids " by J. W. McBain (Routledge and Sons, Ltd., 1932).

temperature, cloudless skies and comparatively low relative humidity during the day. A short note stating some of the results obtained has been published elsewhere [Ramdas and Mallik, 1938] ; in the present paper the results are discussed more fully.

II. Materials and methods

The materials used for this study were :

(*a*) Black cotton soil of Poona.
(*b*) Wheat grain.
(*c*) Paddy grain.
(*d*) *Bajri* grain.
(*e*) *Jowar* grain.
(*f*) *Jowar* leaf (cut into pieces).
(*g*) *Jowar* leaf (entire).
(*h*) *Jowar* stalk (cut into pieces).

All the materials were perfectly dry in the agronomical sense. The specimens were exposed to the atmosphere in small vessels embedded in the soil and their weights determined at desired intervals.

The various conditions to which the specimens were exposed in different experiments were :

(*i*) ' Open and exposed to solar radiaton '
(*ii*) ' Open but shaded from solar radiation ' and
(*iii*) ' In the Stevenson screen at ground level. '

Two sets of experiments were performed. In one set, the specimens were weighed twice daily at 8 A.M. and 4 P.M. consecutively for six days ; this was done with the materials exposed to ' open in sun ' and again with specimens exposed inside a Stevenson screen at ground level. In the other set, on some selected days, the diurnal variation in weights of the different materials was studied by taking two-hourly observations. In every case, simultaneous records of temperature and humidity of the air layers were obtained with an Assmann Psychrometer.

III. Statement of results

A. Maximum and minimum moisture content during the period 23rd November 1937 to 28th November 1937

(*a*) A set of specimens was exposed to the atmosphere in the sun. The specimens were weighed daily at 8 A.M. and 4 P.M. for six days. The values of atmospheric humidtiy at the same hours were also calculated from the dry bulb and wet bulb readings of the Assmann Psychrometer. Table I gives the weights in grams of these materials at 8 A.M. and 4 P.M. from the 23rd to the 28th November 1937.

At the bottom of Table I is given the mean weights of the specimens at 8 A.M. and 4 P.M. as well as the difference between the mean 8 A.M. and mean 4 P.M. weights expressed as percentages of the mean values for the whole day. The results are shown graphically in Fig. 1.

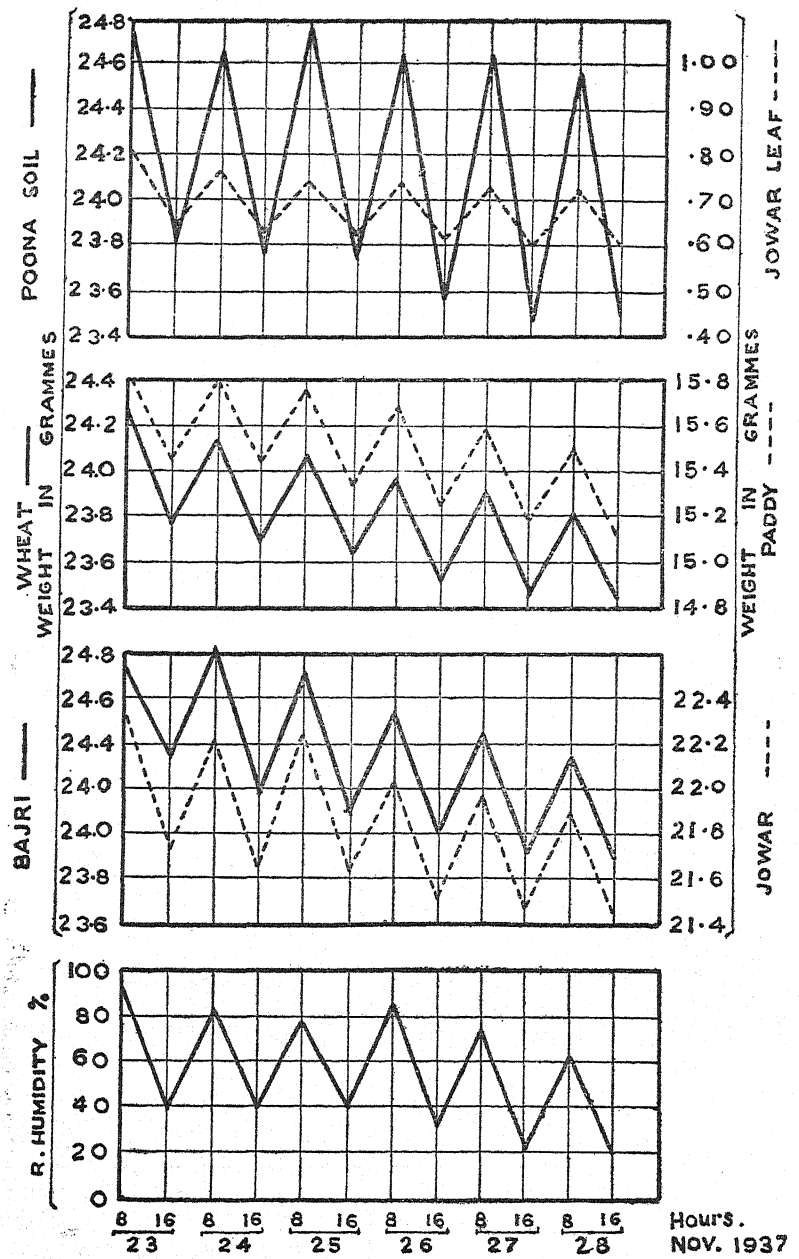

FIG. 1. Weights of samples of Poona soil, *jowar* leaf, wheat, paddy, *bajri* and *jowar* exposed to the open in sun, and values of relative humidity at 0800 hrs. and 1600 hrs. daily from 23rd to 28th November 1937.

(*b*) The above experiment was repeated in January 1938 but this time the specimens were exposed inside a Stevenson screen at ground level.

Table II gives the weights in grams of the materials at 8 A.M. and 4 P.M. from the 17th to the 22nd January 1938.

At the bottom of Table II are given the mean weights of the samples at 8 A.M. and 4 P.M. as well as the differences between these mean 8 A.M. and 4 P.M. weights expressed as percentages of the mean weights for the day. The same results are shown graphically in Fig. 2.

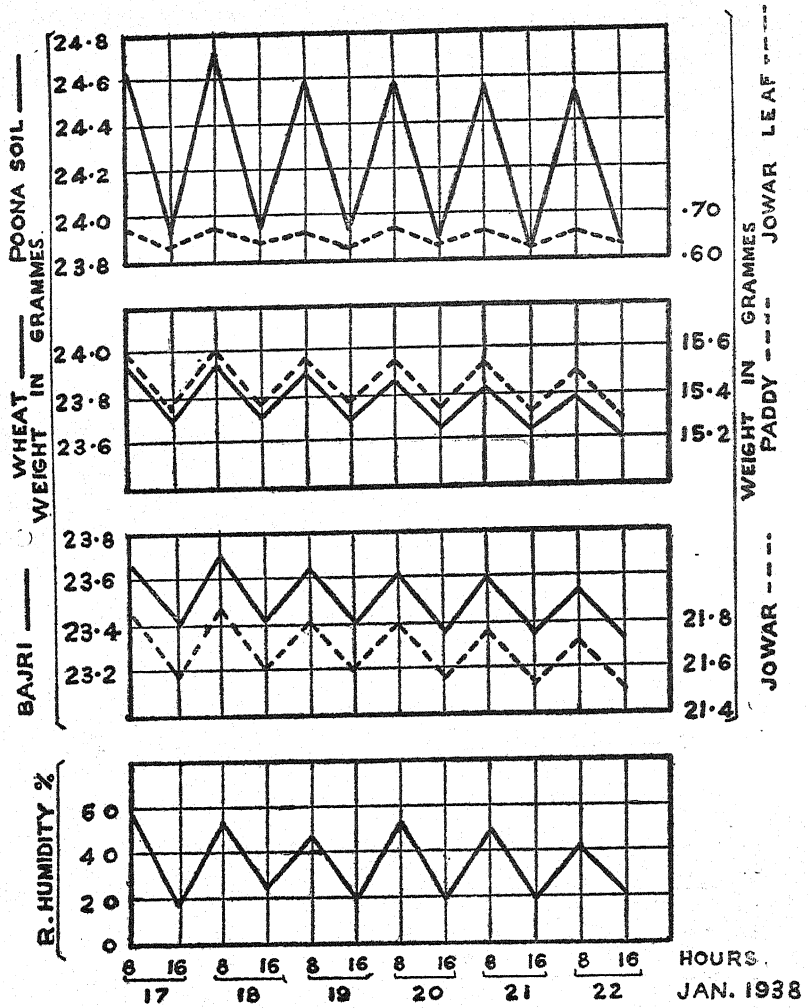

FIG. 2. Weights of samples of Poona soil, *jowar* leaf, wheat paddy, *bajri* and *jowar* exposed inside a Stevenson screen at ground level, and values of relative humidity at 0800 hrs. and 1600 hrs. daily from 17th to the 22nd January 1938.

TABLE I

Open (maximum and minimum weights in grms.)

Date	Time	Weight in grams						Dry bulb (°C.)	Wet bulb (°C.)	Vapour pressure (mm. of Hg)	Humidity (per cent)
		Soil	Wheat (grain)	Paddy (grain)	*Bajri* (grain)	*Jowar* (grain)	*Jowar* (leaf)				
23rd November 1937 .	8 A.M.	24·7343	24·2625	15·8561	24·7349	22·3078	0·8050	10·8	10·0	8·8	91
	4 P.M.	23·8135	23·7737	15·3525	24·3445	21·7319	0·6536	29·8	20·3	13·1	42
24th November 1937 .	8 A.M.	24·6641	24·1430	15·7957	24·8228	22·1974	0·7686	9·8	8·5	7·7	84
	4 P.M.	23·7535	23·6925	15·4309	24·2100	21·6536	0·6364	30·4	20·3	12·8	39
25th November 1937 .	8 A.M.	24·7675	24·0859	15·7743	24·7088	22·2468	0·7440	11·8	9·8	8·1	78
	4 P.M.	23·7307	23·6339	15·3425	24·0970	21·6311	0·6293	29·4	19·8	12·5	41
26th November 1937 .	8 A.M.	24·6605	23·9699	15·6648	24·5328	22·0029	0·7263	10·5	9·2	8·1	85
	4 P.M.	23·5531	23·5215	15·2550	24·0126	21·5196	6·6194	30·2	19·0	10·9	34
27th November 1937 .	8 A.M.	24·6304	23·9115	15·5846	24·4401	21·9651	0·7178	10·2	8·0	6·9	74
	4 P.M.	23·4561	23·4741	15·1915	23·9345	21·4787	0·6006	31·5	17·2	7·6	22
28th November 1937 .	8 A.M.	24·5957	23·8266	15·5077	24·3495	21·8984	0·7150	11·5	8·0	6·3	62
	4 P.M.	23·4323	23·4163	15·1067	23·8735	21·4534	0·5926	33·5	17·5	7·0	18
Mean weight . .	8 A.M.	24·6754	24·0332	15·6972	24·5983	22·1031	0·7461	..	..	7·6	79
	4 P.M.	23·6232	23·5853	15·2800	24·0787	21·5747	0·6219	..	..	10·7	33
Difference between the mean values at 8 A.M. and 4 P.M. expressed as percentages of the mean of the day		4·36	1·88	2·69	2·14	2·42	18·16	..	..	33·88	82·14

TABLE II

Inside Stevenson screen at ground level (maximum and minimum weights in grms.)

Date	Time	Weight in grams						Dry bulb (°C.)	Wet bulb (°C.)	Vapour pressure (mm. of Hg.)	Humidity (per cent)
		Soil	Wheat (grain)	Paddy (grain)	*Bajri* (grain)	*Jowar* (grain)	*Jowar* (leaf)				
17th January 1938 .	8 A.M.	24·6746	23·9379	15·6000	23·6635	21·8378	0·6688	20·5	15·2	10·3	57
	4 P.M.	23·9209	23·6999	15·3583	23·3947	21·5838	0·6333	32·5	17·2	7·1	19
18th January 1938 .	8 A.M.	24·7209	23·9535	15·6143	23·7055	21·8842	0·6736	19·5	13·8	9·0	53
	4 P.M.	23·9477	23·7099	15·3713	23·4211	21·6086	0·6352	31·5	17·8	8·4	24
19th January 1938 .	8 A.M.	24·5957	23·9055	15·5675	23·6359	21·8194	0·6612	20·0	13·2	8·0	46
	4 P.M.	23·9449	23·7031	15·3659	23·4047	21·5950	0·6336	32·0	16·5	6·3	18
20th January 1938 .	8 A.M.	24·5813	23·8621	15·5479	23·6155	21·7998	0·6660	19·8	14·0	9·1	53
	4 P.M.	23·8997	23·6591	15·3351	23·3665	21·5602	0·6340	31·5	16·5	6·6	19
21st January 1938 .	8 A.M.	24·5597	23·8287	15·5279	23·5959	21·7734	0·6644	19·2	13·0	8·1	49
	4 P.M.	23·8701	23·6391	15·3187	23·3527	21·5410	0·6304	32·5	16·8	6·5	18
22nd January 1938 .	8 A.M.	24·5401	23·7843	15·4987	23·5379	21·7230	0·6644	20·5	13·0	7·5	41
	4 P.M.	23·8549	23·6103	15·2853	23·3324	21·5106	0·6288	31·8	17·2	7·4	21
Mean weight . .	8 A.M.	24·6121	23·8787	15·5594	23·6257	21·8063	0·6666	..	..	8·67	50
	4 P.M.	23·9063	23·6702	15·3394	23·3787	21·5665	0·6326	..	..	7·04	20
Difference between the mean values at 8 A.M. and 4 P.M. expressed as percentages of the mean of the day		2·91	0·88	1·42	1·05	1·11	5·23	..	..	..	..

B. Diurnal variation in the weights of specimens (due to exchange of moisture with the air layers near the ground)

(*i*) Specimens were kept in the open exposed to sun's rays and weighed every two hours from the morning of one day to the morning of the next day on two occasions, *viz.*, 17th to 18th and 27th to 28th November 1937. Dry bulb and wet bulb temperatures of the air just above the specimens were also recorded by means of an Assmann Psychrometer.

Tables III and IV give the weights of the materials expressed as percentages of the mean weight as well as the dry bulb and wet bulb readings and the calculated vapour pressure and relative humidity values at two-hourly intervals.

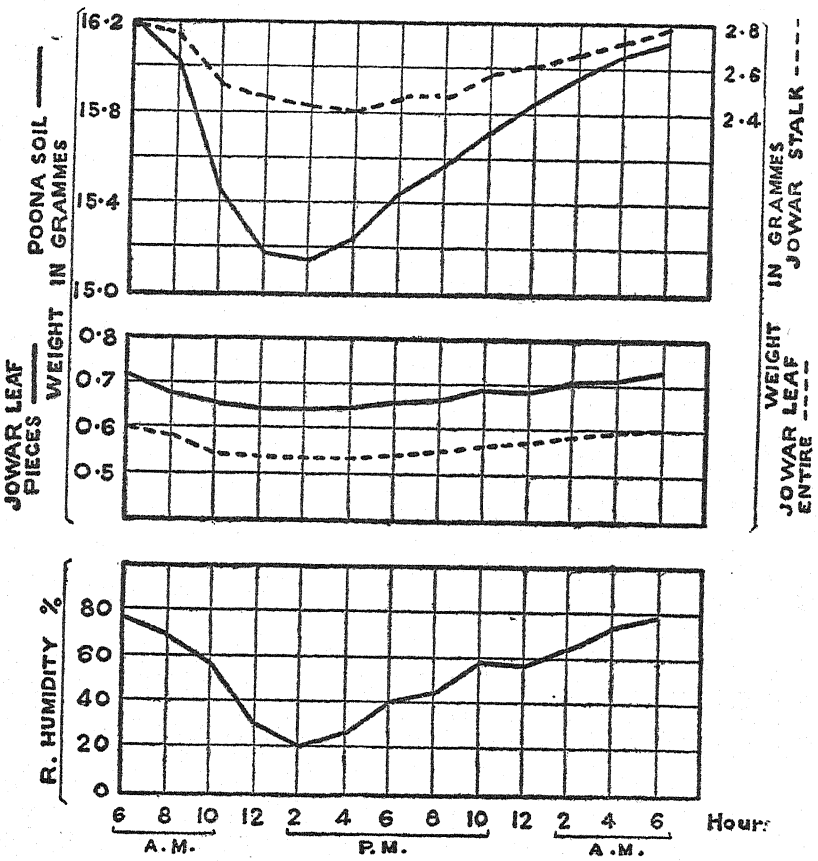

FIG. 3. Hourly variation of the weights of samples of Poona soil, *jowar* stalk, *jowar* leaves cut to pieces and *jowar* leaf entire, exposed to open in sun, and the relative humidity from 0600 hrs. on 17th November to 0600 hrs. on 18th November 1937.

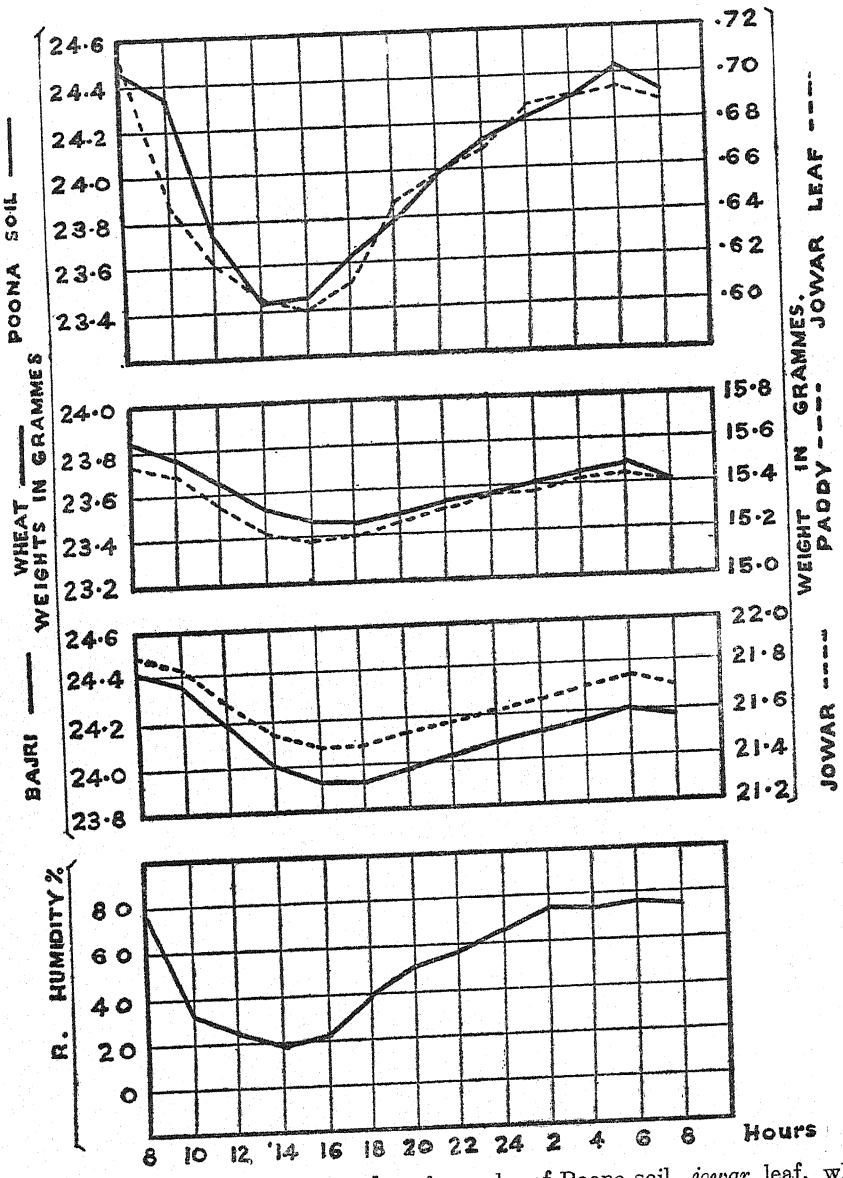

FIG. 4. Hourly variation of the weights of samples of Poona soil, *jowar* leaf, wheat, paddy, *bajri* and *jowar* exposed to open in sun and of the relative humidity from 0800 hrs. on 27th November to 0800 hrs. on 28th November 1937.

TABLE III

Exposed to open in sun (two-hourly observation ; actual weights expressed as percentages of mean weight)

Date	Time (hrs.)	Soil	*Jowar* stalk	*Jowar* leaf cut into pieces	*Jowar* leaf, entire	Dry bulb (°C.)	Wet bulb (°C.)	Vapour pressure (mm. of Hg.)	Relative humidity (per cent)
17th November 1937.	0600	103·3	107·6	105·9	109·5	9·8	7·8	6·9	76
Do.	0800	102·1	105·6	100·5	102·8	13·0	10·0	7·7	69
Do.	1000	98·5	97·6	96·3	95·8	21·8	16·1	10·9	56
Do.	1200	96·7	95·6	94·9	94·4	31·4	19·1	10·4	30
Do.	1400	96·5	93·9	94·9	94·1	36·0	19·8	9·2	21
Do.	1600	97·1	93·1	94·9	94·1	30·8	17·8	8·8	26
Do.	1800	98·4	95·3	96·6	95·5	24·0	15·5	9·0	40
Do.	2000	99·2	95·6	97·7	96·9	18·8	12·2	7·4	45
Do.	2200	100·2	99·5	101·1	99·3	14·2	10·0	7·1	59
Do.	2400	101·2	101·3	101·5	101·3	14·0	9·8	7·0	58
18th November 1937.	0200	101·6	103·0	103·6	103·0	12·5	9·2	7·1	65
Do.	0400	102·4	104·8	105·1	104·7	10·8	8·5	7·2	74
Do.	0600	102·8	107·1	106·9	108·7	9·9	8·0	7·2	79
100 per cent =		15·6926 grms.	2·5861 grms.	0·6803 grms.	0·5739 grms.				
Range of variation (per cent)		6·8	14·5	12·0	15·4				

TABLE IV

Exposed to open in sun (two-hourly observations ; actual weights expressed as percentages of the mean weight)

Date	Time (hours)	Soil	Wheat (grain)	Paddy (grain)	*Bajri* (grain)	*Jowar* (grain)	*Jowar* (leaf)	Dry bulb (°C.)	Wet bulb (°C.)	Vapour pressure (mm. (of Hg)	Humidity (per cent)
27th November 1937.	0800	101·8	101·0	101·3	101·2	101·2	108·5	15·5	13·0	10·0	76
Do.	1000	101·5	100·7	100·9	101·0	100·9	98·7	28·0	17·2	9·3	33
Do.	1200	98·5	100·2	100·0	100·3	100·2	94·5	31·5	18·0	8·7	25
Do.	1400	97·6	99·7	99·2	99·6	99·5	92·4	34·2	18·2	7·7	19
Do.	1600	97·7	99·4	98·9	99·2	99·2	91·5	31·5	17·2	7·6	22
Do.	1800	98·4	99·4	99·0	99·2	99·2	93·2	22·5	14·2	8·0	39
Do.	2000	99·1	99·5	99·4	99·4	99·5	98·5	16·7	11·2	7·2	51
Do.	2200	99·9	99·7	99·7	99·6	99·6	100·3	14·0	9·8	7·0	58
Do.	2400	100·4	99·9	100·0	99·8	99·8	102·1	12·2	9·0	7·0	66
28th November 1937.	0200	100·8	100·0	100·2	100·0	100·0	104·6	10·5	8·2	7·0	74
Do.	0400	101·3	100·2	100·4	100·1	100·2	105·1	9·8	7·6	6·7	74
Do.	0600	101·7	100·3	100·6	100·3	100·5	105·7	9·0	7·0	6·5	76
Do.	0800	101·3	100·0	100·5	100·3	100·2	104·8	14·5	12·0	9·3	75
100 per cent =		24·6730 grms.	23·6109 grms.	15·3831 grms.	24·1116 grms.	21·6496 grms.	0·6563 grms.				
Range of variation (per cent)		4·2	1·6	2·4	2·0	2·0	17·0				

(*ii*) The above experiment was repeated changing the condition of exposure. The specimens were exposed within a Stevenson screen at ground level. Table V gives the two-hourly weights expressed as percentages of the mean weight for the day and the dry bulb, wet bulb, vapour pressure and humidity values.

TABLE V

Inside Stevenson screen at ground level (two-hourly observations, actual weights expressed as percentages of the mean weight)

Date	Time (hours)	Soil	Wheat (grain)	Paddy (grain)	*Bajri* (grain)	*Jowar* (grain)	*Jowar* (leaf)	Dry bulb (°C.)	Wet bulb (°C.)	Vapour pressure (mm. of Hg)	Humidity (per cent)
11th January 1938.	0800	100·8	100·2	100·4	100·3	100·3	100·2	14·5	9·2	6·1	49
Do.	1000	100·8	100·3	100·3	100·3	100·3	98·2	23·5	13·8	7·0	32
Do.	1200	100·0	100·2	100·1	100·2	100·1	98·1	27·8	15·2	6·7	24
Do.	1400	99·2	99·9	99·7	99·9	99·9	98·1	32·5	17·2	7·1	19
Do.	1600	98·8	99·8	99·5	99·7	99·6	97·8	30·5	17·0	7·8	24
Do.	1800	98·6	99·6	99·3	99·5	99·4	97·7	28·2	17·2	9·2	32
Do.	2000	99·1	99·6	99·5	99·6	99·5	98·7	24·5	15·8	9·1	40
Do.	2200	99·6	99·7	99·6	99·7	99·6	99·8	20·5	13·8	8·5	47
Do.	2400	99·9	99·9	99·9	99·8	99·8	100·4	17·5	12·2	8·0	53
12th January 1938.	0200	100·2	100·	100·2	100·1	100·0	101·3	14·8	10·5	7·4	58
Do.	0400	100·5	100·2	100·4	100·2	100·2	102·9	12·2	9·2	7·2	68
Do.	0600	101·2	100·3	100·6	100·4	100·4	103·4	12·0	10·0	8·2	78
Do.	0800	101·2	100·3	100·6	100·4	100·7	103·5	14·2	9·8	6·9	57
100 per cent=		24·2642 grms.	23·7510 grms.	15·4192 grms.	23·4734 grms.	21·6658 grms.	0·6559 grms.				
Range of variation (per cent)		2·6	0·7	1·3	0·9	1·3	5·8				

(*iii*) In this experiment the materials were exposed to open but shaded from the sun.

Table VI gives the two-hourly weights expressed as percentages of the mean weight of the day as well as the dry bulb and wet bulb temperatures and the calculated vapour pressure and relative humidity values.

TABLE VI

Open (in shade) (two-hourly observations ; actual weights expressed as percentages of the mean weight)

Date	Time (hours)	Soil	Wheat (grain)	Paddy (grain)	*Bajri* (grain)	*Jowar* (grain)	*Jowar* (leaf)	Dry bulb (°C.)	Wet bulb (°C.)	Vapour pressure (mm. of Hg)	Humidity (per cent)
4 thMarch 1938	0800	100·3	100·5	100·0	100·2	100·0	103·0	27·1	16·4	8·6	32
Do.	1000	99·1	99·9	99·7	99·7	99·8	101·7	31·7	17·4	7·8	22
Do.	1200	98·0	99·5	99·6	99·3	99·6	98·3	33·5	17·1	6·4	16
Do.	1400	98·0	99·5	99·5	99·2	99·4	95·4	38·5	18·0	5·2	10
Do.	1600	97·7	99·4	99·4	99·0	99·3	95·1	38·0	18·0	5·5	11
Do.	1800	98·3	99·4	99·5	99·3	99·5	96·5	33·0	20·2	11·4	30
Do.	2000	99·6	99·7	99·7	99·6	99·6	96·7	30·3	20·0	12·4	38
Do.	2200	100·6	99·7	99·9	99·8	99·8	97·0	28·0	18·5	11·2	40
Do.	2400	101·1	100·1	100·4	100·2	100·1	99·4	24·5	16·3	9·8	43
5th March 1938	0200	101·5	100·3	100·5	100·6	100·5	101·5	21·7	14·5	8·8	45
Do	0400	102·0	100·6	100·7	101·0	100·8	104·0	19·5	13·3	8·3	49
Do.	0600	102·4	100·8	100·8	101·2	101·1	106·5	17·0	11·3	7·2	50
Do.	0800	101·3	100·5	100·2	100·8	100·4	104·5	23·4	17·3	11·8	55
100 per cent=		24·2104 grms.	23·2003 grms.	25·0679 grms.	22·8563 grms.	21·0889 grms.	0·2110 grms.				
Range of variation (per cent)		4·7	1·4	1·4	2·2	1·8	11·4				

(*iv*) In this experiment two sets were exposed simultaneously, one in the open with the sun's rays falling on the specimens while the other was exposed in the open but shaded from the sun. The specimens were weighed at two-hourly intervals and the readings of the dry bulb and wet bulb temperatures recorded at the same time.

Tables VII and VIII give the diurnal variation in the weights expressed as percentages of the mean weights for the whole day as well as the dry bulb, wet bulb, vapour pressure and relative humidity values.

TABLE VII

Exposed to the open in sun (two-hourly observations ; actual weights expressed as percentages of the mean weight)

Date	Time (hrs.)	Soil	Wheat (grain)	Paddy (grain)	*Bajri* (grain)	*Jowar* (grain)	*Jowar* (leaf)	Dry bulb (°C.)	Wet bulb (°C.)	Vapour pressure (mm. of Hg)	Humidity (per cent
2nd May 1938	0800	105·9	102·5	103·2	104·2	102·7	124·1	36·5	19·0	7·7	17
Do.	1000	103·4	101·5	101·5	101·2	100·7	100·4	38·5	19·5	7·5	15
Do.	1200	97·0	99·7	99·3	99·8	99·8	75·7	43·5	20·5	6·6	10
Do.	1400	94·4	99·2	98·6	98·8	99·2	73·5	43·0	20·0	6·0	9
Do.	1600	94·8	98·8	98·2	98·0	98·7	71·6	42·0	21·2	8·5	14
Do.	1800	96·1	98·7	98·3	97·9	98·6	79·6	35·5	19·5	9·0	21
Do.	2000	97·8	99·0	99·0	98·5	98·9	91·3	31·5	21·5	14·2	41
Do.	2200	99·0	99·3	99·4	99·0	99·2	98·4	28·0	23·0	18·6	65
Do.	2400	100·7	99·5	99·7	99·6	99·5	104·4	25·2	24·0	21·8	91
3rd May 1938	0200	101·7	100·0	100·3	100·2	100·3	114·8	23·0	21·0	20·0	95
Do.	0400	102·5	100·4	100·6	100·7	100·7	120·3	21·5	19·5	18·4	96
Do.	0600	103·3	100·7	100·9	101·1	101·0	123·3	20·5	18·5	15·0	83
Do.	0800	103·2	100·7	100·9	101·0	100·9	123·0	34·5	25·0	19·0	46
100 per cent =		23·0254 grms.	26·5556 grms.	25·6193 grms.	20·5482 grms.	19·3589 grms.	1·3618 grms.				
Range of variation (per cent)		11·5	3·8	5·0	6·3	4·1	52·5				

TABLE VIII

Exposed to the open but always in shade (two-hourly observations ; actual weights expressed as percentages of the mean weight)

Date	Time (hrs.)	Soil	Wheat (grain)	Paddy (grain)	*Bajri* (grain)	*Jowar* (grain)	*Jowar* (leaf)	Dry bulb (°C.)	Wet bulb (°C.)	Vapour pressure (mm. of Hg)	Humidity (per cent)
2nd May 1938	0800	101·9	101·0	101·3	101·4	101·4	106·0	34·5	18·5	8·0	19
Do.	1000	100·2	100·3	100·2	100·5	100·6	99·4	37·5	18·2	6·0	12
Do.	1200	98·3	99·9	99·1	99·6	99·8	98·1	40·8	18·0	4·1	7
Do.	1400	98·0	99·4	98·8	99·2	99·5	93·6	41·0	19·8	6·7	11
Do.	1600	98·2	99·2	98·8	99·1	99·1	98·6	39·5	21·5	10·2	19
Do.	1800	98·8	99·1	99·0	99·0	99·0	99·0	36·5	20·6	10·2	22
Do.	2000	99·2	99·2	99·3	99·3	99·3	99·5	30·0	18·5	10·2	32
Do.	2200	99·6	99·5	99·6	99·6	99·6	99·9	26·5	22·7	18·8	72
Do.	2400	100·0	99·7	100·1	99·9	99·8	100·5	25·0	24·0	21·9	92
3rd May 1938	0200	100·6	100·2	100·5	100·2	100·2	101·0	22·5	22·0	19·6	96
Do	0400	101·3	100·5	100·9	100·6	100·4	101·3	21·5	21·0	18·4	96
Do.	0600	102·0	101·0	101·3	100·9	100·8	101·8	20·5	18·5	15·0	83
Do.	0800	101·8	100·9	101·2	100·8	100·5	101·5	32·0	28·3	23·2	65
100 per cent =		22·8402 grms.	20·5787 grms.	24·3880 grms.	24·0255 grms.	22·7844 grms.	1·1888 grms.				
Range of variation (per cent)		4·0	1·9	2·5	2·4	2·4	12·4				

IV. Discussion

A. Explanation of the results

From the foregoing account it emerges that plant materials exchange moisture with the air layers surrounding them just as the soils do. The curves of diurnal exchange of mositure by plant materials are somewhat similar to that of the soil; both the soil as well as the plant materials lose moisture from morning till afternoon, but regain during the afternoon and night almost all the moisture lost during the day. There is also a striking similarity in the form of the curves of soil and plant materials to that of relative humidity. In other words, as the relative humidity of the air decreases, soil and plant materials tend to give up moisture to the air layers, but when the relative humidity of the air increases, they desiccate the atmosphere by absorbing moisture from the air layers surrounding them.

B. Maximum and minimum moisture content

The epochs of maximum and minimum moisture contents vary slightly in the different cases. In soils the epoch of minimum moisture content coincides with the maximum temperature epoch while the maximum moisture content occurs at the same time as the minimum temperature epoch. In the case of plant materials, except when the materials are exposed inside a Stevenson screen, the maximum moisture content occurs about the same time as the minimum temperature epoch, *i.e.*, at about 6 A.M. The loss of weight from 6 A.M. to 8 A.M., however, is not so well marked as in the case of soil. While the soil starts losing moisture from 6 A.M., the loss of weight from 6 to 8 A.M. is generally not very striking in the case of plant materials. Inside a Stevenson screen the intensity of the phenomenon is less and there is a general lag; the plant materials attain their maximum weight only at 8 A.M., and not at 6 A.M. as in the other cases when exposed to the open.

The minimum moisture content of plant materials, unlike soils, does not occur exactly at the maximum tem perature epoch. In this respect, the plant materials studied show difference even amongst themselves, some showing the minimum weight at about 2 P.M. as the soil does, while others go on losing weight till as late as 6 P.M. It is seen that, in general, plant materials attain their minimum moisture content a little later than the soil, materials like seed grains showing a greater lag than do specimens of dry *jowar* stalk or leaf. Thus, the lag, which is slightly noticeable at the maximum moisture epoch, becomes strikingly apparent (4 hours sometimes) during the minimum moisture epoch. The exchange of moisture between a soil in a finely divided state and the air is more rapid than in the case of the plant materials. In the case of plant materials the process is not so rapid because of the cell walls through which the water vapour has to pass in getting in or going out. The seed-coat of the grains is still less permeable to water vapour than the dry cells of *jowar* leaf or stalk and hence the lag in the epochs of maximum and minimum moisture content is more in the case of the grains than in the case of dry *jowar* leaves and stalks.

C. Diurnal variation in moisture content under different conditions

The difference between the maximum and minimum weights, or the amplitude of variation expressed on percentage basis, is shown in Table IX.

TABLE IX

The amplitude of variation in weight (or the maximum weight minus the minimum weight expressed as a percentage of the mean weight), on different days and under different conditions of exposure

Materials	17th November 1937, exposed to open, in sun	27th November 1937, exposed to open, in sun	11th January 1938, exposed inside S.S. at ground level	4th March 1938, exposed to open, shaded from sun	2nd May 1938, exposed to open, in sun	2nd May 1938, exposed to open, shaded from sun
	1	2	3	4	5	6
1. Black cotton soil.	6.8	4.2	2.6	4.7	11.5	4.0
2. Wheat grains	..	1.6	0.7	1.4	3.8	1.9
3. Paddy grains	..	2.4	1.3	1.4	5.0	2.5
4. *Bajri* grains	..	2.0	0.9	2.2	6.3	2.4
5. *Jowar* grains	..	2.0	1.3	1.8	4.1	2.4
6. *Jowar* leaf cut into pieces	12.0	17.0	5.8	11.4	52.5	12.4
7. *Jowar* leaf entire	15.4					
8. *Jowar* stalk cut into pieces	14.5					

Table IX brings out the following points :—

(1) The diurnal variation is greatest in dry plant materials like *jowar* leaf and stalk and least in the seed grains, while soil occupies an intermediate position in this respect.

(2) The diurnal variation is maximum when the samples are exposed to the sun, and minimum when they are exposed in a Stevenson screen ; when exposed in the open but shaded from sun, the values are intermediate between those in the above two cases.

(3) The effect of season can be studied by comparing column 2 with column 5 as well as column 4 with column 6 where the same materials were exposed to identical conditions in different parts of the year. It becomes clear from the above comparison that the exchange of moisture between soil and plant materials and the air layers surrounding them is greater in the hotter than in the colder parts of the year.

(4) The effect of insolation is clearly brought out by a comparison of columns 5 and 6. The conditions of exposure were similar in all respects except

that in one case the direct insolation was out off. It is seen that just by cutting off the insolation the amplitude of the variation in weights is reduced by about 50 per cent.

Significance of the above results in agricultural practice

The fact that dry plant materials lose moisture during the day and regain during the night may have a bearing on agricultural marketing. From what has been said it is evident that a difference between the maximum and minimum weights (for materials like dry *jowar* stalk and leaf) equivalent to about 10-15 per cent of the mean weight for the day is not unusual when the material is fully spread out. In actual practice grain and fodder are handled in bulk and it is necessary to find out how the diurnal variation of moisture content would alter under those conditions. Further work on these lines will be attempted during the next clear season.

V. Summary and conclusions

(1) It has been shown that dry plant materials exchange moisture with the air layers surrounding them just as the soils do.

(2) The amount of the moisture exchanged is greater than in the soil in the case of materials like dry *jowar* stalk and leaf, while in the case of grains like wheat, paddy, *bajri* and *jowar* the moisture exchanged is less than that by the soil.

(3) The difference between the maximum and minimum moisture content is greatest in materials exposed to open in sun, smaller when exposed to open shaded from sun and least when the specimens are exposed within a Stevenson screen at ground level.

(4) The amplitude of the variation in weight is greater in summer than in winter.

(5) The agricultural bearing of these results has been indicated.

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THE PRACTICAL SIGNIFICANCE OF SOIL SOLUTION STUDIES

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I. Introduction

A few references have been made to the writers direct and sometimes through the Imperial Bureau of Soil Science with regard to the value of displaced solution of soil at its moisture content under normal field conditions. A preliminary survey of the soil solution methods has been made and a few facts about them have been put forth in the following pages.

A ' soil solution ' is obtained from a soil of optimum moisture content by the alcohol displacement method of Parker [1921] and other workers as described later on in the paper. The soil solution isolated by this method is the soil water, containing some colloidal and dissolved salts removed unchanged

from the solid mass of soil and forms, to the best of our knowledge, the medium from which the plant draws its nutrients. An examination of the analyses of these solutions enables us to follow the dynamic changes which take place in soils as a result of irrigational, seasonal or cultural variations.

There is a considerable literature on methods of determining the soil solution. One of the first attempts to separate soil solution without recourse to dilution appears to have been made by Schloesing, [1866, 1870], who attempted to displace the soil solution by gravity with water but the method did not work well with soil containing less than 20 per cent water. Itscherekov [1907] improved on Schloesing's procedure by substituting alcohol for water as the displacing liquid. Parker [1921] made a study of the alcohol displacement method and in comparing ethyl alcohol with other liquids such as acetone, methyl alcohol and water, found that ethyl alcohol was the most satisfactory displacing agent. The writers have found out that methylated or rectified spirit yields equally satisfactory results. The method evidently gives the true soil solution, which is at least twenty times as concentrated as the 1 : 5 water extract, though about ten times as much soil is needed compared with the soil used in an ordinary water extract. The greatest advantage it has over water extracts is that it gives a more correct measure of the material in solution. It being concentrated takes least time for evaporation in all micro-determinations used in the investigations.

Burd and Martin [1923] have carried out tests with the gravity displacement method using water instead of alcohol and, in addition, a positive air pressure up to 100 lbs. per sq. inch, thereby hastening the displacement. Their soils seem to be of simpler types and therefore the displaced solutions obtained by them do not exhibit any wider differences in comparison to water extracts of the corresponding soils. They have, however, concluded that the displaced solutions of the soils eliminate some of the more important objections which have been raised to water extraction of the ordinary type. They have stated that the displacement method has much to recommend it in the study of fundamental investigations of soil chemistry in which soluble constituents are concerned.

Burgess [1922], testing the Lipman's method, isolated the soil solution by direct pressure using soil containers capable of withstanding 100,000 lbs. to the sq. inch and compared the extracted solution with 1 : 5 water extracts. The values for the soil bases and other fertility constituents obtained by him (whether expressed on air-dry or oven-dry soil basis) from 1 : 5 extracts are higher than those found in the soil solution in any individual soil.

Data presented in the following pages will show that the discrepancies between the soil solution and water extracts become still more apparent when soils of different kinds are examined. The value of soil solution methods becomes much more reliable when soils of arid regions are examined.

Eaton and Sokoloff [1935] have shown that the proportional and absolute amounts of sodium present in aqueous extracts of soils are higher than those

in displaced soil solution. The proportion of calcium is lower in extracts than displaced solutions.

The results obtained by the writers are shown in the following pages.

II. Research carried out

The principal object of the investigation has been to examine critically the value of soil solution methods and their application to various soil conditions and plant growth in general. Attempt has been made to employ these methods in the study of base-exchange reactions and subsequently in the investigations into the nature of alkali and saline soils.

The comparative value of the examination of soil solution and of water extracts with different soil-water ratios has been studied particularly in case of natural soils rich in soluble bases and also in case of artificially salted soils.

III. Laboratory methods

(*a*) *General considerations*

If water is placed on top and allowed to percolate through a mass of moist soil in a loose state of aggregation, such as that in which it exists in the field under conditions normal to plant growth, successive portions of the liquid obtained will be decreasingly concentrated until a low and comparatively uniform level is reached. None of these portions can properly be assumed to represent the soil solution and the concentration of the entire solution so obtained will doubtless approximate that of a water extract obtained with similar portions of water and soil.

On the other hand if a similar mass of soil is pressed tightly in a tube container and treated with alcohol or any other displacing liquid the successive portions of solution have equal concentrations until the displacing agent begins to appear. These portions, before getting contaminated or diluted, represent true soil solution.

(*b*) *Method of obtaining soil solution*

The method has been described by Parker [1921, 1927], Flint [1927], Mackness [1932] and others, but as it is not widely practised in India and some other countries, a brief description of the writers' technique is given below :—

A bakelite tube or an ordinary glass percolator $2\frac{1}{2}$ inches diameter is closed by muslin tied over the lower end, and moist soil, about two kilos, is then charged into the tube in small quantities at a time, each small addition being pressed down before the next is added. The tube is made to stand in a rack on a funnel containing filter paper and alcohol (methylated or rectified) poured on to the top of the soil. In a few hours drops of soil solution appear, and are collected in a trap flask attached to the funnel. It is remarkable that the solution is seldom at all muddy, in fact the filter paper is almost unnecessary,

and is preferably omitted in most cases. If it is desired to reduce the amount of alcohol required, the displacement may be started with 200 or 300 c.c. of alcohol and after this has penetrated the soil, water may be added to complete the displacement.

It may be emphasised that the soil solution thus obtained is no way an alcoholic extract, neither does it contain any trace of alcohol when the operation is correctly carried out. If the tubes are allowed to stand too long, then the alcohol begins to come through, and can at once be detected by the characteristic appearance which due to defraction differences accompanies the mixing of an alcoholic drop and water. About 50 per cent of the soil solution is displaceable by this method and the successive portions of the liquid obtained give the same freezing point depression [Parker, 1921], and exhibit a constant composition in successive increments.

The method can be used on all soil classes and at a wide range of moisture contents. It has several distinct advantages over other pressure methods which have been used by other workers. One of the greatest advantages is its simplicity and the fact that it does not require any special apparatus.

Relation between moisture and results of analyses.—The direct results of analyses are expressed in parts of nutrients and other bases per million parts of the solution and this represents the concentration of bases and other nutrients at the time of displacement. Finally, however, all the results are expressed on oven-dry soil basis as parts per million or per hundred. Parker and others have shown that at ordinary moisture contents the concentration of the soil solution is inversely proportional to the moisture content of the soil.

(c) *Methods of analyses*

Methods adopted, except in case of sodium, are those generally in use in the Agricultural Chemistry Department of the Imperial College of Science and Technology, London, for work of this kind, and have been tested over a number of years. An easy and short method for the determination of sodium in soils has been successfully worked out [Mulwani and Pollard, 1937]. Accuracy of each method was tested [Mulwani, 1936] with synthetic soil solutions made prior to the research.

Preparation of solution for the estimation of manganese, calcium, magnesium, etc.—Fifty c.c. of soil solution or water extract are evaporated to dryness in a silica dish with a few c.c. of A. R. hyrodchloric acid. The residue is then ignited thoroughly but not too strongly to burn organic matter completely. Five c.c. of A. R. nitric acid and one drop of hydrochloric acid are added and the whole evaporated again on a steam-bath. It is then digested on the steam-bath with 50 c.c. of water, acidified with a few drops of nitric acid, for fifteen minutes or until the flakes of calcium sulphate dissolve. The digested material is filtered through a fitler No : 40 Whatman paper into a 100-c.c. measuring flask (*A*) and made up to volume.

Manganese.—Five c.c. of test solution from flask (*A*) are pipetted in a 60-c.c. beaker and treated with one c.c. of phosphoric acid and a few crystals of potassium periodate and heated to boiling. After allowing it to stand for five minutes it is heated again, stirring all the time to avoid bumping, and made to 10 c.c. The colour developed in presence of manganese is quickly compared with standard.

Preparation of standard.—Direct use of standard potassium permanganate solution in some cases is unsatisfactory as shade of colour is slightly different from that obtained with test solution. Therefore 10 c.c. of $N/20$ potassium permanganate are acidified with sulphuric acid and reduced with oxalic solution and re-oxidised with phosphoric acid and periodate and finally made to 1,000 c.c. The resulting standard is $N/2{,}000$. ($N/10$ $KMnO_4$ contains 1·10 grm. Mn per 1,000 c.c.). The best part of range is between 1-2 c.c. of $N/2{,}000$ potassium permanganate ; with 3 c.c. the colour is difficult to match.

The comparisons are made in exactly similar specimen tubes etched with a mark at a volume of 10 c.c. and standing on a white tile. In one is placed the test solution diluted to 10 c.c., into the other the standard is run from a graduated pipette until on looking down the tubes the tints are seen to be the same. The final comparison is made with the standard diluted to about 10 c.c. so that equal amounts of manganese will be contained in equal volumes of solution. Following recoveries have been made by this method :—

Quantities of Mn taken	Quantities of Mn recovered
(Mg.)	(Mg.)
0·02	0·020
0·02	0·018
0·02	0·018
0·02	0·022
0·05	0·052
0·05	0·050
0·05	0·050
0·05	0·050
0·10	0·104
0·10	0·104
0·10	0·108
0·10	0·100
0·15	0·156
0·15	0·160
0·15	0·160
0·15	0·158

The results indicate the accuracy of the method in presence of very small amounts of manganese. Colour intensity may alternatively be marked in a Hellige colorimeter.

Calcium.—The presence of manganese exceeding 100 p.p.m. interferes with the determination of calcium. Removal is effected by adding 3 grms. of sodium acetate, a few drops of pure bromine, boiling and filtering.

Fifty c.c. of test solution (A) are taken and after treating with 3 drops of concentrated acetic acid it is diluted to 150 c.c. After heating it to boiling 10 c.c. of saturated ammonium oxalate (this precipitates up to 70 mg. Ca) solution are slowly added with stirring. After heating again for ten minutes it is allowed to stand for four hours. The precipitate is filtered through No. 40 Whatman paper and washed well with hot water, the first two washings being collected with the filtrate for estimation of magnesium. The precipitate is rinsed off with cold water and the paper washed through three times with warm dilute sulphuric acid (5 c.c. concentrated acid plus 100 c.c. water). Further 10 c.c. of 1 : 1 sulphuric acid are added and the whole diluted to 150 c.c. After heating nearly to boiling it is titrated with $N/20$ potassium permanganate (1 c.c. $N/20$ $KMnO_4$=1·002 mg. of Ca). Accuracy of the method is shown below :—

Quantitites of Ca taken	Quantities of Ca recovered
(Mg.)	(Mg.)
1·0	0·96
1·0	0·96
2·0	1·90
2·0	1·94
3·0	2·86
3·0	2·98
4·0	4·08
4·0	4·10

Magnesium [Treadwell and Hall, 1915].—The filtrate and washings are boiled down in a 250-c.c. beaker to a volume of about 50 c.c. and 2 c.c. of 10 per cent diammonium hydrogen phosphate solution are added to the hot liquid and 20 c.c. of 10 per cent ammonia stirred in, rubbing when solution has cooled with a rubber-tipped rod, until the precipitate appears. The beaker with the precipitate is allowed to stand, covered to prevent undue loss of ammonia, in the cold for fourteen hours and then filtered through a No. 42 (or 40) paper, washing twice on the paper with one per cent ammonia. The filtrate and washings could be set aside for sulphate determinations. The precipitate is further washed four times on the paper with 30 per cent alcohol (400 c.c. of rectified spirit made up to one litre) rejecting the washings. The filter paper with the washed precipitate is replaced in the beaker with water and left on the hot plate at 60°C. for fifteen minutes to ensure the removal of the ammonia. After cooling an excess of $N/10$ hydrochloric acid is run into the beaker from a micro-burette, usually 5 c.c. suffice ; 30 c.c. of hot water are poured on and this is followed by three drops of a solution of cochineal (1 part crushed beetles digested with 10 parts of 25 per cent alcohol and filtered). After five minutes the liquid is titrated with $N/10$ caustic soda yellow to purple. A blank is carried out, using a piece of the filter paper for each series of estimations.

(N/10 NaOH × 1·21=mg. Mg)

The method is tested :—

Quantities of Mg taken	Quantities recovered
(mg.)	(mg.)
1.0	1.08
1·0	1.02
2.0	2.06
2·0	2.06
4.0	3.86
4·0	3.91

Iron.—Total iron is determined by the thioglycollic acid method as suggested by Lyons [1927]. The solution is first tested qualitatively. From the intensity of the colour obtained the approximate concentration may be assessed. The dilution is then adjusted to about 1 : 500,000. At this dilution the colours obtained are very easy to match. The solution prepared for testing is then transferred to a test-tube marked at 5 c.c. volume. To this is now added one drop of thioglycollic acid and 0·5 c.c. of concentrated aqueous ammonia. The colour produced is then compared with standards of known concentration by means of a Hellige wedge colorimeter.

Quantities of Fe taken	Quantities of Fe recovered
(mg.)	(mg.)
0.1	0.110
0·1	0.110
0·1	0.098
0.2	0.200
0·2	0.220
0·2	0.190
0.5	0.520
0·5	0.500
0·5	0.490

Aluminium.—Aluminium (1·0-20 p.p.m.) is determined directly with a colorimeter, if the solution is clear, by aluminon method [1936]. Quantities between 0·02 and 1·0 parts per million are best measured by comparison with standard in flat-bottom specimen tubes, using three drops of reagent for 5 c.c. of test solution.

The test solution after treatment with the reagent is made up to a definite volume, say 5 c.c., in a test tube. It is then well agitated, heated for five minutes at 100°C. in an evenly heated water-bath and allowed to cool slowly to room temperature.

However, if the test solution is rich in organic matter and calcium sulphate, a simple pre-treatment of the solution is made. Twenty c.c. of the solution are taken in a small silica basin, dried and ignited very gently. If manganese be present the ash is treated with five drops of hydrogen peroxide,

a few c.c. of water, and 2 c.c. of concentrated nitric acid and dried again, and further heated at 105° C. for twenty minutes or at 165° C. for five minutes. Extraction is made with one c.c. of dilute nitric acid (1 : 50). If calcium sulphate is seen at the bottom, it is removed by filtration or centrifuging.

A Hellige wedge colorimeter is used in most cases.

Iron, if present in great excess, interferes in the determination. The solution containing large excess of iron is treated for about five seconds with hydrogen sulphide before putting in the aluminon reagent. Iron, in this manner, is made absolutely inactive, and the aluminium recation is in no way influenced by hydrogen sulphide.

Preparation of aluminon reagent

Aluminium aurin tricarboxylate	0.75 grm.
Ammonium acetate	200.00 grm.
Gum acacia	15.00 grm.
Conc. HCl A. R.	189.00 c.c.

The components are dissolved separately, the gum acacia being filtered through filter paper. the whole being mixed and diluted to 1,500 c.c.

Standard aluminium solution.—1·3662 grm. of $Al_2(SO_4)_3$. ($18H_2O$, A. R.) crystals are dissolved in a few c.c. of water with 2 c.c. of concentrated hydrochloric acid (A. R.). The volume is made to 500 c.c. This gives 221·2 p.p.m. aluminium. The solution is further diluted twenty-five times to get 8·85 p.p.m. aluminium.

Chenery [1936] has found that free aluminium cannot be detected in normal soil solutions of *p*H values between 6·7 and 7·5 ; within a certain range of *p*H the lower the *p*H the higher the aluminium content ; the concentration of other ions had a marked effect on the aluminium content at any given reaction. Accuracy of the method has been tested (4 replications) :—

Al taken (mg.)	Al recovered (mg.)
0.010	0.010
	0.009
	0.009
	0.010
0.050	0.045
	0.048
	0.054
	0.050
0.100	0.100
	0.095
	0.100
	0.110
0.200	0.220
	0.200
	0.180
	0.180

Alkalinity (*or* Na_2CO_3).—This is estimated by evaporating a certain volume of soil solution to dryness and extracting the residual with a few c.c. of warm water. The titration is made with $N/100$ hydrocholric acid using phenolphthalein as indicator.

1 c.c. $N/100$ HCl=1·08 mg. Na_2CO_3

Total carbonate in soil.—This is determined by Collins' Calcimeter [1906].

Nitrogen.—This exists in three main forms in the soil and its solution ; as ammonia, as nitrate, and as organic nitrogen. For all the nitrogen determinations the micro-Kjeldahl still of Pregl [1937] is used.

Ammonia.—In this case the solution (about 5 c.c.) is distilled in the ordinary way with 10 c.c. of a 10 per cent magnesia suspension into $N/100$ acid. When there was trouble due to frothing in the distillation with magnesia suspension, it was replaced with 3 c.c. of 40 per cent magnesium sulphate solution plus one c.c. of caustic soda 30 per cent solution with 5 per cent sodium thiosulphate. In this way satisfactory results were obtained.

Nitrate.—In actual practice ammonia and nitrate have been determined together and the amount of ammonia found taken off gives the net value for the nitrate present in the sample.

The nitrate is, in the first instance, reduced to ammonia by the iron reduction method. 0·2 grm. of reduced iron Kahlbaum is added to the solution and 2 c.c. of sulphuric acid (1 : 1) are then carefully pipetted in to rinse down all the iron particles adhering to the neck of micro-Kjeldahl flask. The flask is shaken and stood for five minutes. It is then slightly warmed till the digestion is complete and the solution quite clear. The distillation is carried out as above.

Organic nitrogen.—This form of nitrogen as well has been obtained by differnce between total nitrogen and ammonia plus nitrate. In estimating total nitrogen the procedure is similar to that mentioned above up to digestion with reduced iron. Thereafter the material is treated with one drop of sodium selenite or selenium oxychloride and 2 c.c. of concentrated sulphuric acid. The digestion is carried out carefully to avoid bumping. The flask is kept steady by means of a lead ring round its neck. The distillation is carried out with 16-18 c.c. of 30 per cent caustic soda mixed solution into $N/100$ hydrochloric acid. The accuracy of the methods is tested with following results :—

1. Ammonia (*N*) (*A. R. ammonium sulphate solution used*)

Nitrogen taken 0·211 mg. in duplicate.

Nitrogen recovered.

	mg.
(*a*) Distillation with 10 c.c. of 10 per cent MgO suspension . . .	0·205
	0·206
(*b*) Distillation with 3 c.c. of 40 per cent $MgSO_4$ solution with a 1 c.c. of 30 per cent NaOH solution	0·204
	0·207

2. Nitrate (N) (A. R. potassium nitrate solution used).
Nitrogen taken 0·277 mg. in duplicate.

Nitrogen recovered—	mg.
(*a*) Distillation with 10 c.c. of 10 per cent MgO suspension	0·260
	0·272
(*b*) Distillation with 5 c.c. $MgSO_4$ (40 per cent solution) with 6 c.c. of 30 per cent NaOH solution	0·268
	0·279

3. Total nitrogen in three forms :—
Triplicate quantities taken :

	mg.
N (as ammonium sulphate A. R.)	0·106
N (as potassium nitrate A. R.)	0·139
N (as urea pure)	0·233
Total	0·478

Total nitrogen recovered :

mg.
0·482
0·469
0·475

Phosphoric acid.—Phosphate has been determined by Deniges' colorimetric method, using the Hellige colorimeter. The method as recommended by Parker and Fudge [1927] is followed—

Preparation of the solution.—A convenient quantity of soil solution (containing PO_4 from 2 to 14 mg.) is pipetted into a deep porcelain crucible. Two c.c. of dilute nitric acid are added and the solution evaporated on the hot plate at 60°C. The residue is heated very carefully, but sufficiently to render organic matter insoluble. Pre-ignition in an electric oven at 160°-180°C. is preferable when calcium content is high. Risk of explosive ignition of calcium nitrate with organic matter is thus restricted. The material is then taken up in 0·2 c.c. of 5 per cent hydrochloric acid and washed into a measuring flask, adding 0·1 c.c. of molybdic reagent and making up to the mark. If the solution is turbid it is cleared by centrifuging at 3,000 r.p.m. This is then treated with one drop of stannous chloride reagent and the blue colour developed compared with standards similarly treated with molybdic and stannous chloride reagents.

Calibration curves may be prepared from different standards which will afford an easy calculation of result.

Ammonium molybdate reagent.—One hundred c.c. of 10 per cent ammonium molybdate solution are mixed with 300 c.c. of a 50 per cent (by volume) sulphuric acid. The acid must be free from arsenic and the reagent kept in the dark or stored in a brown bottle.

Stannous chloride reagent.—To 0·1 grm. of powdered tin is added one drop of 4 per cent solution of copper sulphate and 2·0 c.c. of concentrated hydrochloric acid free from arsenic. The material is warmed to hasten the reaction and the reagent diluted to 10 c.c. This reagent must be prepared fresh every day.

Standard phosphate solution.—0·1433 grm. of potassium dihydrogen phosphate is dissolved in distilled water and made to 1,000 c.c. in a measuring flask. This is protected with a thin layer of toluene. The solution contains 100 p.p.m. of phosphate. Dilutions are made according to the standard desired.

Potassium.—The silver cobalti-nitrite method for the micro-determination of potassium (0·02 to 0·8 mg.) in soil solutions as worked out by Ismail and Harwood [1937] has been adopted by this department.

The method described has been tested and found to give accurate results for amounts of potassium between 0·02 mg. and 0·8 mg.

In the case of saline soils, which are exceedingly rich in potassium, very small volumes, 0·1 c.c. to 1·0 c.c., are taken for the determination or else the amount of potassium exceeds the range (0·02—0·8 grm.) of determination. It has been found that the chlorine content of the solution, which is easier to know, gives a rough index of the potassium content of the solution. On knowing the chlorine content one could decide the volume of the solution to be taken for the determination of potassium to be within the range of the determination.

From general experience, the following table has been prepared to serve in judging the volume of solution to be employed in the determination :—

Cl p.p.m.	Vol. of s.s. in c.c.
10-25	5
25-100	2
100-250	1
250-500	0·5
500-5000	0·2
5000-10000	0·1

However, these should be considered as very approximate figures, as it is observed that salt-affected unleached soils are exceedingly high in potash content, while leached soils are very much poorer in the same.

Data to show the accuracy of the method of potassium determination (K as KNO_3 A. R.)

Quantities of K taken in duplicate (mg.)	Quantities of K recovered (mg.)
0·02	0·022
	0·020
0·05	0·054
	0·049
0·10	0·092
	0·120
0·20	0·204
	0·202
0·40	0·390
	0·398
0·80	0·756
	0·786

K as (KNO_3 A. R.) in presence of 5 c.c. of Slough (England) soil solution.

Quantities taken of K in duplicate	Quantities* of K recovered
(mg.)	(mg.)
0·05	0·044
	0·048
0·10	0·102
	0·096
0·20	0·188
	0·196

Hydrogen-ion concentration (p*H*).—Quinhydrone electrometric method has been followed in the case of Devon soils, while colorimetric method has been adopted in the case of alluvial calcareous soils from Sind.

(1) The quinhydrone electrode portable apparatus is described and designed by Brown and Broom [1929].

Standards were :—

0·2 N sodium acetate

0·2 N acetic acid

9 c.c. acid plus 1 c.c. acetate gave 3·72 *p*H

1 c.c. acid plus 9 c.c. acetate gave 5·57 *p*H

The order of accuracy of the method was 0·02 *p*H.

(2) For the determination of *p*H values of soil solutions colorimetrically two apparatuses have been used :—

(*a*) The B. D. H. capillator, and

(*b*) The Lovibond Comparator.

Soil moisture.—Moisture is determined by heating 5 to 10 grm. of soil in a well ventilated electric or air oven at 105°C. for twenty-four hours.

Sodium was determined by the colorimetric method mentioned in a previous publication [Mulwani and Pollard, 1937]. This procedure is further simplified in case of saline soils as the solutions of these soils are absolutely free from acidity and contain little phosphate or organic matter to interfere in the precipitation of sodium uranyl zinc acetate. Treatments with alcoholic zinc acetate-hydroxide as given by McCance and Ship [1931] has been found unnecessary in case of saline soils.

*These quantities are net after deducting the quantity of K in the soil solution.

A comparison of original McCance and Ship's detailed and the authors' simplified methods yielded following results :—

Mg. of sodium taken	Mg. of sodium recovered			
	(i) McCance detailed method	Mean value	(*ii*) Simplified method	Mean value
0·0393	0·038 (2)		0·039 (6)	
	0·039 (8)		0·038 (9)	
	0·038 (8)		0·039 (8)	
	0·039 (6)	0·039 (1)	0·039 (3)	0·039 (4)
0·0786	0·077 (7)		0·078 (2)	
	0·078 (4)		0·078 (2)	
	0·078 (1)		0·079 (0)	
	0·079 (0)	0·078 (3)	0·078 (6)	0·078 (5)
0·1180	0·117 (1)		0·117 (6)	
	..		0·118 (8)	
	0·117 (7)		0·118 (6)	
	0·118 (0)	0·117 (6)	0·117 (8)	0·118 (2)
0·1570	0·15 (7)		0·15 (8)	
	0·15 (8)		0·15 (6)	
	0·15 (7)		0·15 (8)	
	0·15 (8)	0·15 (8)	0·16 (0)	0·15 (8)
0·2360	0·23 (6)		0·23 (4)	
	0·23 (4)		0·23 (8)	
	0·23 (5)		0·23 (8)	
	0·23 (5)	0·23 (5)	0·23 (8)	0·23 (7)

Thus it is seen that the simplified method is quite perfect.

IV. Soils used in the investigation

Soils from Devon (England) and Sind (India) were examined through the consideration of their displaced solutions and corresponding water extracts. A brief description of each soil is given below :—

(*a*) *Devon*

The soil was originally received from Greenwell Farm and was under grass. High ammonia content (Table VII) was perhaps due to long storage. Mechanical composition of the soil is given :—

	per cent
Course sand	13·42
Fine sand	29·40
Silt	22·55
Clay	19·75
CO_3	<0·20
Organic matter	9·30
Loss by solution	1·45

Composition of the soil solution (Table VII) shows that excepting potash, which is low, the soil is fairly rich in other constituents. It is acidic in reactions.

(*b*) *Sind*

The soils, which are both made and maintained by the huge River Indus, are fertile. They are deep, calcareous alluviums, mostly loamy, with a low water-holding capacity and a very low humus content. As a result of successive deposition and erosion by the Indus, the proportions of sand and clay vary considerably within even the smallest areas. The soil profile is undefined and structureless. A striking feature of these soils is their lack of uniformity over any distance. A number of pits, which have been dug to a depth of 5 to 6 feet in a series of plots show distinct layers of soils deposited one over the other, the order of deposition being often different in different pits. In one of the pits the thickness of different layers and their arrangement was as follows [Tamhane and Daji, 1929] :—

Layer	Texture	Composition (per cent)	
		Silt+clay	Fine sand
First layer (0—7 in.)	Clay loam	46·90	53·10
Second layer (7—19 in.)	Loam	30·41	69·59
Third layer (19—25 in.)	Clay loam	50·77	49·23
Fourth layer (25—46 in.)	Sandy loam	14·38	85·62
Fifth layer (46—72 in.)	Stiff clayey	79·70	20·30

The thickness and the nature of the layers, however, varies in different pits. Besides these differences of texture the soluble salt content (comprising mostly NaCl and Na_2SO_4) of each layer varies very markedly [Tamhane and Mulwani, 1934].

It is obvious that the results obtained at one place cannot be applied to another place. This does not, however, mean that there is nothing of general importance. There are many things which are, in spite of the heterogenity of the soil, found to be common in movement and storage of water and bases in these soils. The soils in general are slightly alkaline in reaction. Iron, aluminium and manganese, therefore, do not appear in the solution of these soils. Phosphate also remains mostly in insoluble form.

The rainfall is scanty, the average being 3 inches per annum, which is mostly received during the summer months in light showers. Under these soil and climatic conditions, the agriculture therefore depends entirely upon irrigation throughout the country.

V. Experimental

Attempt has been made to study the possibility of applying soil solution methods to investigations of causes of soil exhaustion, deterioration and subsequently find out methods of soil amelioration and general improvement to permit normal plant growth.

The work for the sake of simplicity and convenience has been divided into the following main sections :—

(*a*) Co-relation between soil productivity and its soluble constituents. Soil conditions, under normal and poorer growths of ordinary farm crops, like wheat (*Triticum sativum*), *Jowar* (*Sorghum Vulgare*) and cotton (*Gossypium neglectum*), were examined through considerations of soil solutions, water and hydrochloric acid-extracts.

(*b*) Comparison of displaced soil solutions and water extracts in the saline and alkaline soils, and

(*c*) base-exchange reactions in soils.

Each section is dealt with separately :—

(*a*) *Soil solution* vs. *soil productivity*

Soils of known productivity in the field which were free from injurious salts were taken up and examined through considerations of soil solutions, water extracts and chemical analyses respectively. The results of the analyses have been put up in Tables I (*a*) and I (*b*).

TABLE I (*a*)

Composition of soil solution and water extract from soils of known productivity

Soil	Displaced soil solution: (lbs) Yield per plot (1/16 acre) Grain	Displaced soil solution: (lbs) Yield per plot (1/16 acre) Total dry wt.	Displaced soil solution: 1 Total soluble matter (per cent)	2 Ca (per cent)	3 Mg (per cent)	4 Na (per cent)	5 K (per cent)	NO_3 (per cent) mg.	PO_4 p.p.m.	*p*H	1 : 5 water extracts: 1 Total soluble matter (per (cent)	2 Ca (per cent)	3 Mg (per cent)	4 Na (per cent)	5 K (per cent)	NO_3 (per cent)	PO_4 p.p.m.	*p*H
											(i) Wheat							
A	21	71	0·022	0·002	0·003	0·003	0·001	T	T	8·2	0·08	0·006	0·002	0·002	0·002	T	3·0	7·4
B	38	100	0·054	0·006	0·004	0·006	0·002	0·10	T	7·8	0·120	0·008	0·003	0·003	0·002	0·10	3·6	7·6
C	47	112	0·061	0·006	0·004	0·005	0·002	0·24	0·1	7·6	0·092	0·014	0·004	0·005	0·003	0·2	3·8	7·3
D	57	135	0·104	0·008	0·005	0·004	0·002	0·55	0·2	7·6	0·150	0·012	0·004	0·004	0·005	0·7	3·8	7·4
E	73	172	0·118	0·012	0·004	0·003	0·003	0·62	0·2	7·4	0·162	0·022	0·006	0·007	0·004	0·7	4·0	7·3
F	82	210	0·120	0·015	0·003	0·003	0·004	1·10	0·3	7·2	0·150	0·020	0·006	0·008	0·005	1·2	4·0	7·6
											(ii) Jowar							
G	33	516	0·022	0·003	0·003	0·004	0·001	T	T	8·0	0·08	0·006	0·001	0·002	0·004	T	2·0	7·4
H	46	619	0·08	0·008	0·004	0·008	0·002	0·20	0·1	7·9	0·22	0·008	0·006	0·006	0·008	0·2	2·2	7·6
I	64	677	0·16	0·010	0·004	0·005	0·004	0·50	0·2	7·4	0·28	0·022	0·005	0·008	0·012	0·5	3·6	7·6
J	83	816	0·16	0·018	0·003	0·003	0·005	1·50	0·3	7·4	0·20	0·020	0·004	0·006	0·010	1·8	3·2	7·4
											(iii) Cotton							
	Seed cotton																	
K	52		0·20	0·006	0·006	0·012	0·002	0·40	T	8·4	0·40	0·040	0·005	0·02	0·01	0·8	4·4	7·4
L	97		0·21	0·019	0·003	0·004	0·006	1·40	0·4	7·4	0·25	0·022	0·004	0·006	0·01	1·2	3·4	7·6

T=Traces.

(Columns 1-5, figures expressed on oven-dry soil)

All soils are more or less of similar texture.

TABLE I (*b*)

Ca-Na, Ca-Mg and Na-K ratios in displaced soil solutions and corresponding water extracts from soils of known productivity (Table Ia)

	Displaced soil solution			1 : 5 water extract		
	Ca : Na	Ca : Mg	Na : K	Ca : Na	Ca : Mg	Na : K
Soils under wheat						
A	0·66	0·66	3·00	3·00	3·00	1·00
B	1·00	1·50	3·00	2·66	2·66	1·50
C	1·20	1·50	2·50	2·80	3·50	1·66
D	2·00	1·60	2·00	3·00	3·00	0·80
E	4·00	3·0	1·00	3·10	3·66	1·75
F	5·00	5·0	0·75	2·50	3·66	1·60
Soils under jowar						
G	0·75	1·0	4·00	3·00	6·00	0·50
H	1·00	2·0	4·00	1·33	1·33	0·75
I	2·00	2·5	1·25	2·75	4·40	0·66
J	6·00	6·00	0·60	3·33	5·00	0·60
Soils under cotton						
K	0·50	1·00	6·00	2·00	8·00	2·0
L	4·75	6·33	0·66	3·66	5·50	0·6

Table I (*a*) presents values for total soluble matter and *p*H ; soluble plant food ingredients and bases both from soil solution and water extracts are also given. The data is self-explanatory. Definite co-relation between the composition of soil solution and plant performance is established. Water extracts on the other hand fail to give any information about the soil productivity. Taking each determination separately it is observed (*i*) that the increased total soluble matter as represented by the displaced soil solution agrees closely with the corresponding increases in the yield of crops whereas no such agreement is exhibited by the water extract of these soils. Water extracts in general represent larger amounts of soluble matter. (*ii*) Calcium of the soil solution increases with the soil productivity whereas calcium of extracts is somewhat misleading, *e.g.*, soil (K) which is inferior to (L) has yielded higher calcium in extract ; similar discrepancies are shown by the extracts in (E) and (F) soils and also in (I) and (J). (*iii*) Increase of potassium in soil solution corresponds with the increase in the yield of crops

whereas extracts fail to show such regularity. (*iv*) Though the differences between the magnesium contents are not remarkable it is observed that its ratio with calcium (Ca : Mg) in soil solutions as shown in Table I(*b*) is a good indicator of soil fertility. Calcium-magnesium ratio of the soil solution increases with the soil fertility. This is not seen in case of water extracts. (*v*) Sodium of the displaced solution tends to decrease with the richness of the soil. Water extracts show higher sodium content. Sodium values help in forming useful ratios such as calcium-sodium and sodium-potassium in soil solutions, the former increasing with increase in fertility and the latter decreasing. Water extracts are less reliable in this connection. (*vi*) Nitric nitrogen, both in case of soil solutions and extracts, shows higher with higher yields. Water extracts appear to draw out more of nitrate. (*vii*) Soil solution phosphate figures agree with the productivity of the soil, whereas the water extracts fail to show such relationship. On the other hand larger quantities of phosphates are shown by the extracts than what actually are available for the use of plants in the soil. (*viii*) *p*H values of the soil solutions yield very useful information and agree with calcium-sodium ratio. Water extracts on the other hand show great discrepancies about it. With a view to judge the soil solution data as shown in Table 1 (*a*) and compare it with the standard chemical analyses all the twelve soil samples (A-L) were subjected to various fertility constituent determinations, such as total nitrogen, potash, phosphoric acid and lime content. The results of analyses, as shown in Table II, fail to give any useful information with regard to the crop-yielding capacity of the soil.

TABLE II

Chemical analyses of (A-L) soils for total soil-fertility ingredients

Soil	Total nitrogen per cent	K_2O per cent	P_2O_5 per cent	Ca per cent
A	0·06	0·5	0·15	6·92
B	0·05	0·7	0·15	5·89
C	0·05	0·5	0·24	7·14
D	0·04	0·6	0·18	5·08
E	0·06	1·1	0·32	7·06
F	0·06	0·7	0·25	6·24
G	0·05	0·6	0·18	6·22
H	0·06	0·4	0·08	5·88
I	0·05	1·4	0·31	7·20
J	0·06	0·9	0·22	6·02
K	0·06	1·3	0·42	7·18
L	0·06	1·0	0·38	6·82

Total nitrogen was determined by Kjeldahl-Gunning Jodlbaner process.

Even the poorer soils appear to be fairly rich in the main reserved plant food ingredients. This data does not exactly inform us which of the soils lack in available plant food material. The soil solution data seem to rightly apply to the immediate productivity of the soils.

(*b*) *Composition of displaced soil solution and water extracts in the saline and alkaline soils*

It is customary in detecting excessive salinity in saline and alkaline soils of various countries to utilise 1 : 5 water extracts for the purpose and to set out as limits of safety the values as under :—

Total soluble solids not more than 5,000 p.p.m. on dry soil.

NaCl not more than 2,000 p.p.m.

Ca not less than 500 p.p.m. of which not less than 100 p.p.m. occurs as carbonate.

As noted in the introduction to this paper, considerable divergencies are probable between the amounts of soluble matter in soils determined by means of soil extracts and those determined with soil solutions. In order to have more definite idea of these divergencies in case of alkali and saline soils three typical salt-affected soils were examined and the data obtained is shown in Table III.

TABLE III

Composition of displaced soil solution and of water extracts of varying soil-water ratios in (*a*) *alkaline soil,* (*b*) *medium alkaline soil and* (*c*) *saline soil*

	Soil : water ratio	1 Total soluble matter	2 Ca	3 Mg	4 Na	5 K	6 Na_2CO_3	7 PO_4	Ca : Na ratio	Ca : Mg ratio	Na : K ratio	*p*H
(*a*) Alkaline soil	Soil solution at 18 per cent moisture . 100 : 18	25,015	105	120	4,280	318	864	T	0·02	0·87	13·5	9·8
	1 : 1 . .	25,218	220	125	4,018	444	401	0·2	0·05	1·76	9·1	9·1
	1 : 2·5 .	36,714	650	156	3,945	616	45	1·2	0·16	4·17	6·4	8·5
	1 : 5 . .	42,516	2,284	202	3,902	892	*Nil*	2·2	0·58	11·30	4·4	7·6
	1 : 10 .	46,412	2,842	226	3,864	926	*Nil*	4·0	0·73	12·6	4·2	7·6
(*b*) Medium alkaline soil	Soil solution 100 : 15	18,370	124	118	3,892	298	212	0·4	0·03	1·05	13·06	9·2
	1 : 1 . .	25,672	427	124	4,064	388	84	0·4	0·15	3·44	10·5	8·9
	1 : 5 . .	32,544	2,596	216	3,964	578	*Nil*	2·0	0·65	12·02	6·9	7·5
	1 : 10 .	38,484	3,016	248	4,118	784	*Nil*	4·0	0·73	12·16	5·3	7·4
(*c*) Saline soil	Soil solution 100 : 16	13,029	141	133	3,773	275	63	0·2	0·04	1·0	13·7	8·9
	1 : 1 . .	12,760	554	80	3,537	394	*Nil*	0·2	0·16	7·0	9·0	7·8
	1 : 5 . .	21,820	2,164	140	3,537	570	*Nil*	4·0	0·61	15·5	6·2	7·6
	1 : 10 .	31,550	4,068	158	3,520	737	*Nil*	6·0	1·17	25·5	4·6	7·6

(Figures (1 to 7) expressed as parts per million on oven-dry soil)

With the widening of the soil-water ratio there is a marked increase in the apparent values of the soluble calcium, potassium, phosphate and total soluble matter, a less marked increase in magnesium and no change in sodium. The values for base ratios, calcium-sodium and calcium-magnesium, increase and those for sodium-potassium decrease rapidly as the proportion of water used is increased.

A further point of interest is that high alkalinity due to sodium carbonate shown in soil solution analyses disappears in the soil extracts. Presumably at the greater dilution precipitation of calcium and/or magnesium carbonate takes place ; also the high calcium content of the water extract tend to suppress dissociation of the sodium clay and the consequent formation of sodium carbonate. The phosphate of the extracts is correspondingly higher than that of the soil solution.

There seems no continuity between the variations in values obtained with the different soils in water extracts and that appearing in the soil solutions.

The combined experience of other workers in this department and elsewhere points out to the fact that the proportionality between values obtained with the soil solution and those with any particular soil-water ratio differ from soil to soil and in the same soil as a result of different cultural or irrigational treatments.

It seems, therefore, that the safety limits used in practice of water extracts are purely empirical, and their application to different soils is of doubtful value. The divergence between the *p*H and sodium carbonate content of soil solutions and of extracts may also lead to erroneous conclusions. Sodium carbonate and *p*H values of the soil solutions clearly indicate the dynamic aspects of saline and alkaline soils. Soil (*a*) which has shown higher sodium carbonate and *p*H values is evidently a sodium or alkaline soil and has persisted to remain barren even after heavier applications of lime and water in the field trials. Soil (*c*) with lower sodium carbonate and *p*H value is a saline soil and has been fully reclaimed on mere flooding with irrigation water. Soil (*b*) is a medium alkali type and has been reclaimed with great amount of difficulty and is yielding poorer crops.

It is thus observed that it is clearly impossible to get this information from water extracts which do not show any differences with regard to degree of alkalization.

It is, however, suggested that the re-adjustment of base-exchange equilibrium occurring during the preparation of soil-water extracts could easily be compared with that taking place during irrigation and leaching. This matter will more comprehensively be dealt with later on.

Analyses of water extracts prepared with a series of different soil-water ratios may therefore serve to forecast the results of irrigation with varying amounts of water.

(c) *Base-exchange reactions in soils*

The importance of exchangeable bases in soil needs no emphasis. It is well known that a large amount of food material in soils for the use of plants resides in the exchangeable complex, and some of the most fertile soils are those rich in exchangeable bases. The knowledge of the nature and amount of exchangeable bases is of prime importance in all studies of the soil conditions and plant growth.

These reactions are clearly understood in healthy and non-saline soils and several methods have been accurately fixed up for the determination of exchangeable bases. But these methods do not work well in case of saline and alkaline soils. This has also been supported by Hissink [1938]. The main reason has been the practice of determining exchangeable bases in such soils by subtracting the water-soluble bases, as found for example in aqueous extracts, from total bases which results in serious error. It should never be assumed that the water extract results are applicable to ordinary field conditions. It has been clearly shown in Tables I and III and elsewhere that the water extracts do not always give a true picture of the soil conditions and in many cases the analyses are not sufficiently reliable for accurate interpretations. The proportional and absolute amounts of bases in water extracts are quite different from those of the displaced solutions [Burgess, 1922]. This fact has to be remembered at all stages in base exchange studies.

As mentioned before, Eaton and Sokoloff [1935] showed that the proportional and absolute amounts of sodium present in aqueous extracts of their soils were higher than those in displaced soil solutions. While the proportion of calcium is lower in extracts than in displaced solutions. The most satisfactory explanation of this phenomenon given is that a cation-exchange reaction takes place when the water-soil ratio is increased. In this exchange, calcium enters the absorbing complex and sodium is liberated.

However, in the case of saline or alkaline soils containing calcium carbonate, as shown in Table III, the amounts of calcium tends to increase with the dilution. New calcium is liberated into solution on dilution from the soil complex, whereas sodium which is in excess is not much affected.

Such reactions appear to remain depressed under optimum or lower moisture content of the soil as shown by the previous data and as also seen from the following experiment.

A healthy normal soil was taken up and treated with 1·0 per cent sodium chloride and equivalent amounts of sodium sulphate (1·22 per cent) and sodium carbonate (0·91 per cent) to find out the influence of these salts on the soil through considerations of the differences in the displaced soil solutions and corresponding water extracts with 1 : 5 soil-water ratio.

Batches of two kilos of moistened soil were mixed in 1·0 per cent sodium chloride, 1·22 per cent sodium sulphate and 0·91 per cent sodium carbonate respectively and stored in lightly covered basins for a week to ensure that

a condition of base equilibrium was attained. In general practice, soils containing 0·5 per cent sodium chloride or sodium sulphate yield satisfactory crops after heavy irrigations. Those containing 1·0 per cent and upward are definitely harmful. Sodium carbonate is injurious even in very small amounts. The results obtained from the soil solutions and from water extracts with 1 : 5 soil-water ratio are shown in Table IV.

TABLE IV

Base-exchange reactions in soil on application of salts and water

(Figures expressed as p.p.m. on dry soil ; pH values and Ca : Na ratio)

	Soil	Soil : water	Total soluble matter	Ca	Mg	Na	K	Ca : Na ratio	Na_2CO_3	*p*H
A	Original (untreated) .	Displaced soil soln..	1,116	145	32	31	46	4·7	*Nil*	7·3
		1 : 5 extract . .	1,946	248	58	55	94	4·5	*Nil*	7·4
B	Same with 1 per cent NaCl	Displaced soil soln.	10,990	178	40	3,934	59	0·04	T	7·6
		1 : 5 extract . .	14,486	1,582	96	3,640	508	0·43	*Nil*	7·4
C	With 1·22 per cent Na_2SO_4 (equivalent of 1 per cent NaCl) .	Displaced soil soln.	12,880	171	42	3,918	62	0·04	T	7·5
		1 : 5 extract . .	16,265	1,565	102	3,690	550	0·42	*Nil*	7·4
D	With 0·91 per cent Na_2CO_3 (equivalent of 1·0 per cent NaCl) .	Displaced soil soln.	10,010	68	24	3,925	54	0·017	8,992	11·9
		1 : 5 extract . .	14,208	102	60	3,070	486	0·03	6,014	9·2

The data presented under A in Table IV show that the extract brings out comparatively larger total soluble matter and other bases even from untreated healthy soil. These reactions, due to base-exchange phenomenon, are very much accelerated on extraction with sodium chloride and sodium sulphate. Calcium and potassium represented by the extract are about nine times higher than that of soil solution with sodium chloride or sodium sulphate (B and C). The increase in calcium on extraction correspondingly increases calcium-sodium ratio. Na reactions could be examined by substituting ammonium salts instead of sodium salts in the base exchange. Hissinks method is based on these principles of base exchange but applies only to soils which are free from salts.

(D) Solubility of calcium and magnesium, however, remains depressed on treatment with 0·91 per cent sodium carbonate both in displaced soil solution and in extract. This is due to double reaction, *viz.*, usual displacement of calcium and magnesium in first stage and subsequent precipitation of these with the excess of sodium carbontate in second stage. This is confirmed by reduction in sodium carbonate, calcium-sodium ratio and *p*H of water extracts.

This double reaction of sodium carbonate was critically examined through considerations of displaced soil solutions and water extracts of the same healthy soil on treatment with smaller amounts of sodium carbonate. The soil was

treated with 0·01, 0·10, 0·2 and 0·3 per cent sodium carbonate respectively and kept moist exactly as done in previous case. Results of analyses as given in table V clearly show that sodium carbonate added to the soil is more or less completely recovered in all cases of displaced soil solutions. Water extracts fail to give fully the amounts of all the sodium carbonate added to the soil. It is evident that sodium carbonate up to certain limit is precipitated by the displaced lime on extraction. Calcium displaced in sodium carbonate treatment up to 0·1 per cent is more than sufficient to precipitate all this amount of sodium carbonate. Consequently the water extract with 0·1 per cent sodium carbonate treatment neither shows any presence of sodium carbonate nor any rise in *p*H. Excess of sodium carbonate, however, depresses the solubility of calcium or partly utilises the displaced lime in its partial precipitation. This double reaction is therefore responsible for the reduction in recovery of sodium carbonate in water extracts.

TABLE V

Effects of different concentrations of Na_2CO_3 on normal soil

(*Figures expressed as p.p.m on dry soil*)

Treatment	Soil : Water	Total soluble matter	Ca	Mg	Na	K	Ca : Na ratio	Na_2CO_3	*p*H
(*i*) Original healthy soil .	Displaced Soil Soln.	1,116	145	32	31	46	4·7	*Nil*	7·3
	1 : 5 extract .	1,946	248	58	55	94	4·5	*Nil*	7·4
(*ii*) Same with 0·01 per cent Na_2CO_3 . . (*i.e.*, 100 p.p.m.)	Displaced Soil Soln.	1,186	130	30	79	50	1·7	89	8·2
	1 : 5 extract . .	1,224	890	74	83	114	10·7	*Nil*	7·3
(*iii*) With 0·1 per cent Na_2CO_3 . . . (*i.e.*, 1,000 p.p.m.)	Displaced Soil Soln.	2,054	114	30	482	50	0·24	951	9·4
	1 : 5 extract . .	2,482	1,497	98	448	290	3·34	*Nil*	7·7
(*iv*) With 0·2 per cent Na_2CO_3 . . . (*i.e.*, 2,000 p.p.m.)	Displaced Soil Soln.	3,078	110	30	914	52	0·12	1,900	10·1
	1 : 5 extract . .	4,418	224	70	865	310	0·26	804	8·8
(*v*) With 0·3 per cent Na_2CO_3 . . . (*i.e.*, 3,000 p.p.m.)	Displaced Soil Soln.	4,024	88	28	1,227	52	0·07	2,832	10·9
	1 : 5 extract . .	5,892	108	66	1,025	396	0·11	1,704	9·2

It is interesting to note that the displaced soil solution presents true condition of the degree of salinization or alkalization, calcium-sodium ratio and *p*H values which will not be possible with water extracts.

These base-exchange reactions suggested to know exactly the Ca status in soils in absence or presence of salts. An attempt has therefore been made to find out exchangeable calcium in saline and non-saline soil through considerations of soluble calcium both in soil solutions and water extracts. Three soils ;—one normal, second artificially salted and the third natural alkaline

soil were taken up for the study and the data obtained were given below in Table VI.

TABLE VI

Comparison of soil solutions and water extracts in the determination of exchangeable Ca in

(*a*) *normal healthy soil* (*Table IV*).

(*b*) *artificially salted* (*1 per cent Nacl*) *soil* (*Table IV*).

(*c*) *Natural alkaline soil* (*Table III*).

(*i to iii expressed as p.p.m. ; iv as m.e. per 100 grm. of soil.*)

		(*i*) Soluble Ca	(*ii*) Total (Sol+Ex)	(*ii-i*) (*iii*) Exch Ca	(*iv*) Exch Ca M. E.
Soil (*a*)	Displaced soil soln.	145	1,810	1,665	4·16
	1 : 5 Extract	248	..	1,582	3·95
Soil (*b*)	Displaced soil soln.	178	1,810	1,632	4·08
	1 : 5 Extract	1,582	..	228	0·57
Soil (*c*)	Displaced soil soln.	105	3,025	2,940	4·82
	1 : 5 Extract	2,248	..	741	0·18

The data amply manifests that the reserve calcium is liberated from the soil and comes in solution due to base exchange on treatment with a sodium salt. This reaction is very much enhanced in presence of larger quantities of water. In 1: 5 extract of the artificially salted soil (*b*) we observe that the exchangeable calcium is reduced from 4·08 to 0·57 m.e. per 100 grms. of soil. This reduction is evidently due to the liberation of absorbed calcium in the soil and going in solution due to the presence of salt and water. Deterioration of some of the healthy soils on salt intrusion occurs mainly due to this phenomenon. Calcium is leached out from the soil on flooding or irrigation resulting into sodium soils. Unless large reserves of finely and well distributed calcium carbonate are present the soil becomes practically unfit for cultivation and plant growth.

Addition of soluble salts of calcium like calcium sulphate or calcium chloride may at larger expenses recoup the lost calcium and help in reclamation of the deteriorated soils. Prevention is, therefore, always better than cure. The essential feature in the management of such irrigated lands are firstly the exclusion of sodium salts and secondly judicious use of irrigation water.

In the final series it was sought to find out the influence of pure sodium chloride in different amounts on a healthy soil from Devon (England) through the consideration of the differences in the displaced soil solutions from the original soil and that from the salted samples. Figures of analyses obtained are shown below in Table VII.

TABLE VII

Davon soil

(p.p.m. on oven-dry soil and pH values)

Treatment	Ca	Mg	Na	K	Fe	Al	Mn	PO_4	*p*H	NH_3
A—Original soil (untreated)	36·1	1·7	30·4	6·8	*Nil*	1·7	6·0	1·96	5·9	29
B—With 0·5 per cent NaCl	340·6	258	1,013	36·37	*Nil*	14·5	76·5	*Nil*	5·52	84
C—With 1·0 per cent NaCl	398·0	344	2,085	39·4	*Nil*	52·4	99·3	*Nil*	5·15	93
D—With 1·5 per cent NaCl	512·6	348	3,192	40·8	*Nil*	52·4	128·0	*Nil*	4·90	95

Same expressed as equivalents p.p.m.

	Ca	Mg	Na	K
A—Original soil	1·8	0·14	1·32	0·174
B—With 0·5 per cent NaCl . . .	17·0	21·30	44·0	0·938
C—With 1·0 per cent NaCl . . .	20·0	28·40	90·6	1·010
D—With 1·5 per cent NaCl . . .	25·6	28·76	139·0	1·043

It will be seen in general that the bases are brought into solution by sodium chloride ; *p*H values that are lowered down are due to, in the opinion of the writers, the following reaction :

H-complex of the soil+NaCl→HCl+Na-complex.

Increase in the dose of sodium chloride brings out an increased amount of calcium though it is not proportionate. In the case of potassium and magnesium the reaction is practically complete with one per cent sodium chloride.

Examination of the sodium values shows that in the three salted soils the sodium concentration of the soil solution is increasing regularly with the amount of sodium chloride added. Soil being acidic and unsaturated appears to adsorb sodium of sodium chloride. Even with the heavier treatments sodium still continues to disappear from solution since each increase of sodium chloride (0·5 per cent) represents 86 equivalents of sodium per million parts of dry soil, whereas the correspondent treatments in soil solution concentrations are equivalent to 47 equivalent parts per million of dry soil.

Exchange reactions on magnesium and potassium appear to have ceased with the 1·0 per cent application. With 1·5 per cent salt, displacement of calcium from the clay complex and possibly to some extent from calcium carbonate present in the soil still continues together with that of aluminium and manganese. A more deep seated reaction than that of ordinary base exchange is thus indicated.

*p*H changes are such as would be anticipated as the result of neutral salt additions to a normal soil of low calcium carbonate content.

The disappearance of phosphate from the soil solution of salted soils is noteworthy, as also is the increased proportions of Al appearing in solution. It seems possible that aluminium liberated from the soil complex by the neutral salt action is causing the removal of phosphate as aluminium phosphate. As will be seen later, small amounts of phosphate in the soil solution are characteristic of Sind saline soils examined, but in this case there is a larger proportion of (approximately 10 per cent) calcium carbonate present. The Devon soil used in these tests has less than 0·2 per cent calcium carbonate. In order to facilitate compa rison with previous soils it was desirable to ascertain whether calcium carbonate affected the action of sodium chloride in decreasing the solubility of phosphate a further sample of the soil was mixed with 10 per cent of calcium carbonate and 1·0 per cent sodium chloride and the soil solution examimined after several days. Values obtained were :—

	Fe	Al	Mn	PO_4	*p*H
Untreated soil . . .	*Nil*	1·8	8·0	2·1	5·2
Soil with 10 per cent $CaCO_3$ and 1 per cent NaCl .	*Nil*	*Nil*	*Nil*	*Nil*	6·8

In the presence of calcium carbonate, therefore, salt suppresses the solubility of phosphate and also that of aluminium and manganese. This condition is also similar to that of Sind saline soils. Further, in view of these results it appears unlikely that aluminium is concerned in the reduced phosphate solubility under saline conditions.

VI. Discussions

The main object of the research was to make a detailed inquiry into the status of soil solution and to study the application soil solution methods to investigation of causes of soil productivity, exhaustion or deterioration. Little or no information is available as to the composition of soil solutions from saline and arid region areas, and it appeared likely that the examination of soil solutions would yield information of value in this respect. The validity of this assumption is examined. For reasons already set out, the divergence, between the composition of soil solution and corresponding water extracts, is a matter of some importance if the values of soils are assumed to represent the actual soluble salt status of soils at the time of examination. This divergence has been established in a number of normal and salt affected soils.

Data now presented show that in saline soils the difference between soil solutions and that of water extracts is particularly marked in the case of certain of the most important constituents, *e.g.*, calcium-sodium ratios, alkali carbonates, phosphate, etc.

As a result, the actual status of the soil indicated by the two methods is vitally different. It is therefore believed that for this and other purposes as shown in foregoing pages the soil solution presents the better basis for consideration. The water extract method probably gives information from which a forecast of the effects of leaching may be deduced.

In order to obtain information as to the primary effects of the presence of sodium chloride in soils on the composition of the soil solution, a normal English (Devon) soil was examined. As was to be expected the presence of sodium chloride resulted in the displacement of the principal bases, and their appearance in the soil solution in increasing proportion. The difference in the case of magnesium was very marked and the high values obtained conform to the recognised importance of magnesium salts in the toxicity of saline soils. The complete disappearance of phosphates from the soil solution following salt treatment falls into line with the fact, observed later on, that in soil solutions from saline soils little or no phosphate occurred.

The appearance of iron, aluminium and manganese in large proportions in the soil solutions at first suggested that perhaps fixation of phosphate by one of the bases explained its disappearance from the soil solution. However, the natural saline soils examined contained 9-10 per cent calcium carbonate, whereas the salted Devon soils had very little reserve calcium carbonate. Addition of calcium carbonate with sodium chloride to this soil resulted, as before, in the disappearance of phosphate from the soil solution, but the latter now contained no iron, aluminium, or manganese. In the natural saline soils no iron, aluminium or manganese occur in the soil solutions. It seems unlikely, therefore, that the depression of phosphate solubility in saline soils can be attributed to the fixation of this kind. It is more probable that fixation is related to the high Ca content of the saline soils.

Soil solution evidently helps in the determination of soil fertility and plant performance. Ratios such as calcium : sodium , calcium : magnesium and sodium : potassium as presented by soil solution are valuable ; *p*H value, phosphate and nitrate content of the soil solution yield most satisfactory data on this point. No such agreement is exhibited by water extracts or even chemical analyses of soil for its total fertility constituents.

Referring to saline and alkaline soils a further point of interest is that alkalinity due to the presence of sodium carbonate shown in soil solution analyses disappears in the water extracts. Presumably at the greater dilution precipitation of calcium and/or magnesium carbonate takes place ; also the high calcium content of water extract tends to suppress dissociation of the sodium clay, and the subsequent formation of sodium carbonate. This came to be true and was supported by evidence obtained under study of (*c*) 'Base-exchange reactions' and the data presented in Tables IV and V. Divergence between the *p*H and sodium carbonate content of soil solutions and of extracts may therefore lead to erroneous conclusions. Sodium carbonate and *p*H values of the soil solutions clearly indicate the dynamic aspects of saline and alkaline soils. There seems no continuity between the variations in values obtained with the different soils from water extracts and that appearing in their displaced solutions.

The combined experience of other workers and the writers points out to the fact that the proportionality between values obtained with soil solution and those with any particular soil water ratio differ from soil to soil and in the same soil as a result of different cultural and irrigational treatments.

It seems, therefore, that the safety limits used in practice of water extracts are purely empirical and their application to different soils is of doubtful value.

Comparison of soil solution and water extracts in the determination of exchangeable calcium and in examination of deteriorated soils has been made. Soil solution as said before gives the most reliable information.

In general the action of salt and/or water in these experiments shows no exception to the fundamentals of base exchange phenomenon.

VII. Summary

No attempt will be made to give a detailed summary of all the findings of the present investigation, the writers being content with drawing attention only to the main conclusions.

Description of a soil solution obtained from a soil of normal moisture content under field conditions is given. In comparison with the usual water extracts (1 : 1, 1 : 5, 1 : 10, etc.) the authors are very definitely of the opinion that the displaced soil solution offers the most reliable information as to the water soluble constituents of soil. Addition of a relatively large quantity of water to a soil must inevitably involve a very considerable shift of the solid-liquid equilibrium, and data obtained from extracts are referable only to the conditions of extraction and have no bearing on the solid-liquid equilibrium under normal field conditions. The nature and proportion of the dissolved constituents as represented by the soil solution and extracts do not show any parallelism in any individual soil. The discrepancies between the two methods become still more apparent when soils of different kinds are examined.

The composition of the displaced soil solution gives more or less a true representation of the liquid phase in soil and therefore forms the nutrient medium of the crop. The relative merits of the soil solution, water extract and acid digestion method in determination of soil nutrient values have been discussed.

Methods of obtaining the soil solution and estimating various bases, fertility ingredients and soil reactions (pH) on micro-scale, as followed in the investigation, have been examined and described.

Possibility of applying soil solution methods to the examination of alkaline and saline soils has been established. Use of water extracts in assessing the proportions of harmful salts is definitely unsatisfactory.

Determination of exchangeable calcium in alkaline and saline soils, soil deterioration in general, has been done through consideration of the displaced soil solution and the water extract in which also the soil solution method establishes its superiority. In general the action of salt and water in these experiments shows no exception to the fundamentals of base-exchange. Use of water extracts may help in forecasting the effects of leachings.

The soil solution approach to the study of some phases of plant nutrition, salt tolerance, and soil behaviour under field conditions, therefore offers some promise and seems worthy of investigation in this connection.

The two minor disadvantages of the soil solution methods are (1) the time required for displacement and (2) the necessity of using a large soil sample than is required by the water extraction and other methods. The time factor is, however, best controlled by experience and care in packing the soil. About ten times (1·5 kilos approximately) as much soil must be used as is required for 1 : 5 water extract. These minor disadvantages are, however, greatly compensated by the recent advances in the development of micro-analyses which offer promise in this direction. A material reduction in the quantity of solution required in the laboratory greatly minimises most of the practical difficulties and also remarkably reduces the time required for evaporation in all the determinations.

VIII. Future scope of work

Authors suggest the use of soil solution methods to students of physical and bio-chemistry in the investigations of—

(1) several soil defects co-related with some of the common diseases of the farm crops,

(2) the movement of soil bases and fertility ingredients,

(3) soil reactions and losses due to drainage,

(4) nutrient requirements of crops, etc.

Acknowledgment.

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COLLOID CONTENT AND THE HYGROSCOPIC POWER OF SOILS

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MANY of the important phenomena taking place in the soil are in a large measure due to its colloids. In the present paper a critical study has been made of two important methods of estimation of total colloids in soils comprising both the organic and inorganic colloids. The first section of this paper deals with the results of this work. In section II the soil-moisture relationships for a number of soils containing varying amounts of colloidal material are discussed.

I. ESTIMATION OF TOTAL COLLOIDS

Experimental

The two methods of estimation of total colloids that have been studied are the Hydrometer method due to Bouyoucos [1928] and the Adsorption or Ratio method [Davis, 1925]. In the Hydrometer method dispersion was effected by means of a stirrer worked by a friction driven motor at about 3,000 revolutions per minute after the addition of normal potassium hydroxide solution for deflocculating the colloids. Fifty grams of soil were treated with 350 c. c. of water containing five c. c. of normal potassium hydroxide. A period of thirty minutes was used for complete dispersion. The suspension was made up to 1,000 c. c. and the reading of the hydrometer and the temperature were noted.

In the Ratio method, the adsorption of water vapour by the soils, when exposed to 3·3 per cent sulphuric acid in vacuum desiccators kept at a constant temperature of 30°C. in a thermostat for ninety-six hours, was determined.

Pure colloid was isolated by means of the Sharple's Centrifuge (Cenco, catalogue C. 227, p. 182) from a few soils and the adsorption by the pure colloids was also studied. The centrifuge was hand-driven, the speed of the vertical bowl being about 25,000 revolutions per minute. The suspension of

the colloid passing through the centrifuge was then filtered through a Pasteur-Chamberland filter and the colloid retained by the filter collected [Gile *et al.*, 1924].

The percentage of colloids obtained by both the methods together with the sum of the fine fractions, clay and silt as determined by the International method [Keen, 1931] are given below in Table I [Rao, 1936].

TABLE I

No.	Source	Depth (inches)	Colloids by Hydrometer (per cent)	Absorption at 98 per cent relative humidity moisture (per cent)	Colloids by adsorption (per cent)	Clay (per cent) International method	Clay and silt (International method) (per cent)	Percolation c. c. per hour
1	Adutural . . .	9—24	11·0	12·6	42·0	44·3	59·5	*
2	Anakapalle . .	6—12	12·6	6·7	22·3	19·9	32·1	*
3	Coimbatore (Cotton Breeding Station).	6—18	18·3	6·3	21·0	24·3	30·6	22·0
4	Hagari (West) . .	12—24	35·9	17·9	59·7	45·2	74·2	3·7
5	Hosur F. No. 41 .	6—18	27·7	7·4	24·7	28·6	35·8	16·4
6	Koilpatti (Red) .	9—21	27·1	11·2	37·3	39·4	41·7	23·6
7	Palakuppam . .	6—18	31·3	10·0	33·3	35·1	38·5	*
8	Palur . . .	8—21	17·8	5·8	19·3	17·0	24·3	*

*Not determined.

Percolation rates have been studied for some of these soils by the method due to Puri [1929] with the modification that brass cylinders were used in place of the glass ones described by him. The average values of the percolation rates are included in Table I. They do not show any simple relationship with the clay or the sum of clay and silt. A rapid fall in the percolation rates with increase in the fine fractions was, however, noticeable in the case of Coimbatore, Hagari and Hosur. This lack of correlation between the amount of clay and the rates of percolation is obviously due to the difference in nature and composition of the soil colloids.

Assuming that soils containing 40 per cent and below as the value of the fine fractions, *i.e.*, clay and silt together, to be ' light ' soils and those having above 50 per cent ' heavy ' soils from the results in Table I it appears that :

(*a*) for heavy soils the Hydrometer method gives a lower value of the colloid content than the Ratio method while for light soils, the results obtained by the two methods agree fairly well with the exception of the soil from Anakapalle.

(*b*) the value of the total colloids obtained by the Adsorption method is much higher than the clay fraction in the case of a heavy soil like Hagari, the value of the clay fraction being 45·2 per cent while that of the colloid content is 59·7 per cent. The dispersion of the colloid in the International ammonia method of mechanical analysis is possibly incomplete and a portion of the silt

fraction is composed of aggregates of colloidal material. Hence the phenomenon of adsorption is shared by what is termed 'silt' in the International method. Alway [1925] believes that a portion of the colloids may be contained even in the sand fraction.

(*c*) the amount of water vapour absorbed by one gram of pure colloid extracted as described above by Sharple's Centrifuge from the black soils of Hagari and Coimbatore is found to be 0·29, a value in agreement with that reported by Robinson [1922] for other types of soils.

Of all the soils examined, the black cotton soils of Hagari and Nandyal show the highest absorption at the different relative humidities (*vide intra*). These soils contain the highest amount of colloid material—a great part of which is inorganic in character.

Further work on the chemical composition of the colloidal material isolated from various soils is in progress.

II. Hygroscopic capacity of soils

The hygroscopic absorption of water by soils which is dependent upon the colloidal content has been studied for some soils at relative humidities of 10, 25, 50, 75, 90 and 98 per cent by the Vacuum desiccator method [Puri *et al.* 1925]. Air-dried soils passed through 2 mm. sieves were exposed in squat form weighing bottles in vacuum desiccators containing suitable mixtures of sulphuric acid and water [J. Ind. Eng. Chem., 1921] to give the required humidities. The period of exposure was ninety-six hours at 30°C. The figures for the moisture absorption for the soils at the different relative humidities are given in Table II.

Table II

No.	Source	Depth (inches)	Moisture absorption at relative humidity					
			10	25	50	75	90	98
1	Aduturai . .	0—9	2·0	3·0	5·1	6·7	9·8	10·6
2	Anakapalle . .	0—6	1·5	2·2	3·5	4·5	6·0	7·0
3	Hagari (West) . .	0—12	3·3	4·9	7·4	9·6	11·6	15·0
4	Hosur F. No. 41 .	0—6	0·5	0·8	1·2	1·6	2·8	5·4
5	Nandyal . . .	0—12	3·2	4·9	7·6	9·6	12·0	14·9
6	Palur . . .	0—8	0·5	0·7	1·1	1·3	1·7	2·4
7	Shemmankuliyur (Coimbatore Dt.)	0—9	0·4	0·5	1·0	1·4	2·2	2·9

In Fig. 1, the values of the hygroscopic capacities of the different soils are plotted against relative humidities.

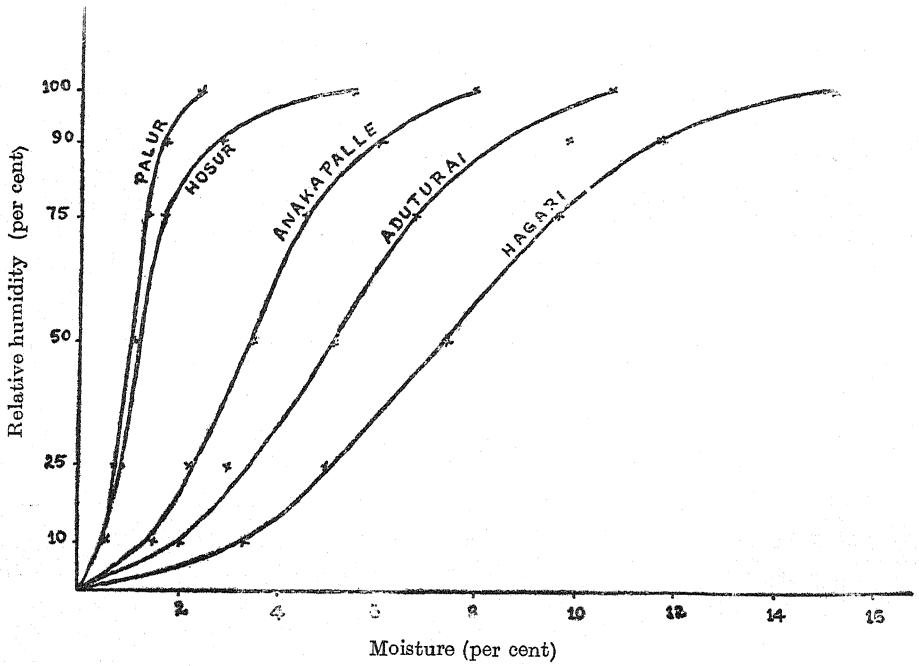

FIG. 1. Vapour pressure curves

(The values for soils Nos. 5 and 7 are not plotted as they overlap with those for soils Nos. 3 and 6 respectively.)

The general shape of the curves is similar for the different soils. They are linear for all soils between 10 and 75 per cent relative humidity. Beyond 75 per cent relative humidity, the curves take a sharp bend tending to become parallel to the moisture axis at higher humidities. The general gradient decreases with increase in the value of the fine fractions.

Progressive absorption of water vapour by soils has been studied at 90, 95 and 100 per cent relative humidities for samples of the Hagari Black soil (East block) taken from plots subjected to normal method of cultivation and from plots which received farmyard manure at the rate of 50 lbs. nitrogen per acre. The moisture content at different periods is given in Table III.

TABLE III

Progressive absorption

Treatment	Layer	Days							
		0	1	2	4	8	12	16	20
At 90 per cent relative humidity									
Control	0-1 ft.	7·73	9·57	10·15	10·67	11·10	11·27	11·32	11·35
	1-2 ft.	8·37	10·08	10·76	11·44	11·90	12·12	12·19	12·21
Farmyard manure . .	0-1 ft.	7·68	9·49	10·21	10·79	11·22	11·39	11·49	11·50
	1-2 ft.	8·20	10·15	10·92	11·62	12·09	12·30	12·38	12·40
At 95 per cent relative humidity									
Control	0-1 ft.	7·65	9·95	10·76	11·66	12·60	13·09	13·45	13·52
	1-2 ft.	8·50	10·69	11·66	12·71	13·68	14·33	14·73	14·83
Farmyard manure . .	0-1 ft.	7·87	10·27	11·15	12·04	12·99	13·62	13·89	13·99
	1-2 ft.	8·41	10·90	11·95	13·00	13·96	14·67	15·09	15·19
At 100 per cent relative humidity									
		0	1	2	3	5	9	13	17	21
Control . .	0-1 ft.	6·52	9·38	10·53	11·20	11·96	12·65	13·65	14·18	14·47
	1-2 ft.	7·11	10·03	11·35	12·16	13·12	14·01	15·08	15·70	16·08
Farmyard manure	0-1 ft.	6·77	9·63	10·81	11·53	12·39	13·39	14·16	14·68	14·98
	1-2 ft.	7·18	10·08	11·44	12·24	13·24	14·15	15·11	15·70	16·04

The absorption of moisture by the soils follows an exponential relationship of the following type :—

$$M = K\,(1 - ae^{-bt})$$

where M is the mositure absorbed by the soil in a period t, K, a and b are constants depending on the nature of the soil and e the base of the Naperian logarithm. The rate of absorption is high at the beginning tending to become constant with increasing time. The results emphasise the importance of specifying the period of exposure whenever 'hygroscopic capacity' of soils is considered.

In Fig. 2 the theoretical values of the moisture content at different periods calculated from the above equation and the observed values are plotted against time. The agreement between the observed and calculated values is closer at lower humidities than at 100 per cent relative humidity. But there is little doubt that the type of absorption follows the exponential relationship given above even at 100 per cent relative humidity.

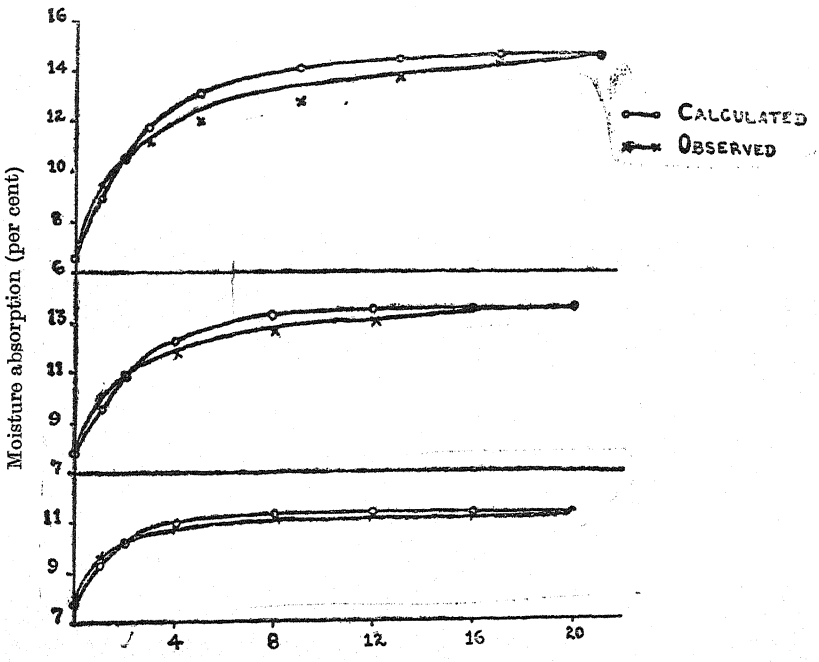

FIG. 2. Progressive absorption of moisture

It is interesting to note that at all the humidities investigated, the soil from the second foot layer invariably absorbed more moisture than that from the first foot layer.

The soil profile at Hagari is fairly uniform to a depth of three feet, with no appreciable variations in the physical composition. The following average values of the mechanical composition by the International ammonia method [Rao, 1937] show that the observed differences in the hygroscopic capacity of the different layers cannot be accounted for by differences in the fine fractions of the soil.

TABLE IV

International ammonia method

	0-1 ft.			1-2 ft.		
	A	B	C	A	B	C
Clay . .	44.9	45.1	45.1	45.3	45.6	45.0
Silt. . .	17.1	17.4	17.3	19.7	17.1	16.6
Fine sand .	15.7	16.4	16.7	15.5	15.5	15.8
Coarse sand .	17.3	16.9	16.6	14.8	16.6	17.7
Loss by solution	5.0	4.2	4.3	4.7	5.2	4.9

(A=Control, B=Bunded, C=Ploughed and bunded)

Similarly the SiO_2/R_2O_3 ratios calculated from the authors' figures for chemical composition of the profile down to three feet [Rao, 1936] were 5·72, 5·94 and 5·44 for the layers 0-1 ft., 1-2 ft. and 2-3 ft., respectively. It is clear from these ratios that the top three feet of the profile is uniform and the possibility of the differences in the hygroscopic capacity being due to lower clay and silt values is eliminated. The decrease in the hygroscopic capacity of the surface soil was thought to be due to the fluctuations in the weather conditions which directly influence the top soil, which is subjected more frequently to alternate heating and wetting than the lower layers. The dehydration of the soil colloids is partly irreversible and hence the absorptive capacity of the surface soil is diminished.

Effect of alternate heating and wetting on the absorptive capacity of the soil.— In order to study the effect of alternate heating and wetting of a soil on its absorptive capacity, the absorption of normal soils for water vapour was compared with that of soils alternately saturated with water and dried. The experimental procedure was as follows :—

After obtaining the normal absorption of the soil, an equal weight of water was added. The soil was allowed to air-dry for two days. It was then dried at 100°C. for about ten hours and equal weights of water added and the soil air-dried for two days. This treatment was repeated five times after which the absorption values were again determined. Similar studies were made by Brown and Byers [1935] on soil colloids extracted from some American soils. The absorptive capacities of some soils treated as above at 75, 99, and 100 per cent relative humidities are given in Table V.

TABLE V

Hygroscopic absorption (period of exposure 5 days)

Depth (inches)	100 per cent relative humidity		99 per cent relative humidity		75 per cent relative humidity		Ratio of 100 per cent to 75 per cent	
	A	B	A	B	A	B	A	B
0-6 .	14.78	13.39	14.07	13.70	8.52	7.89	1.74	1.69
6-12 .	15.73	13.90	15.27	14.11	9.29	8.28	1.69	1.68
12-18 .	16.03	13.85	15.33	14.17	9.38	8.23	1.75	1.68

(A =Before treatment, B =After treatment. The figures are the averages of duplicates which agree among themselves.)

At each of the humidities examined there is evidence for the loss in hygroscopic capacity of the soils when alternately wetted and dried. The decrease in the absorptive capacity of the soils after treatment suggests that the lower absorptive capacity of the top layer, observed in the field, is due to the diurnal variation of temperature as well as the fluctuations in weather conditions to which it is exposed.

The exact effect of soil mulch in field operations is yet a matter of discussion. Besides destroying weeds, soil mulch may be beneficial to the extent that it exposes to the air, a layer of soil which is capable of better absorption than the top layer which is constantly exposed to changes in weather conditions. This fresh layer is able to dehydrate the air in immediate contact to a greater extent than the old layer.

Summary and conclusions

1. Two methods of estimating the amount of total colloids in soil have been critically examined with regard to their suitability for some South Indian soils. It was found that the Ratio or Adsorption method gave higher values of the colloid content than the Hydrometer method.

2. Percolation rates did not show any direct correlation with 'clay' or 'clay and silt' as determined by the International ammonia method.

3. The absorption of moisture at different relative humidities has been studied for different soils.

4. The amount of moisture absorbed at a given relative humidity varies with time according to an exponential law of the type :

$M = K(1 - ae^{-bt})$, where t is the time in days and K, a and b are constants.

5. The moisture retentive capacity of soil has been found to be reduced by alternately heating and wetting it. The bearing of this result on the low moisture retentive capacity of the surface soil compared to that of the subsoil observed in this work has been discussed.

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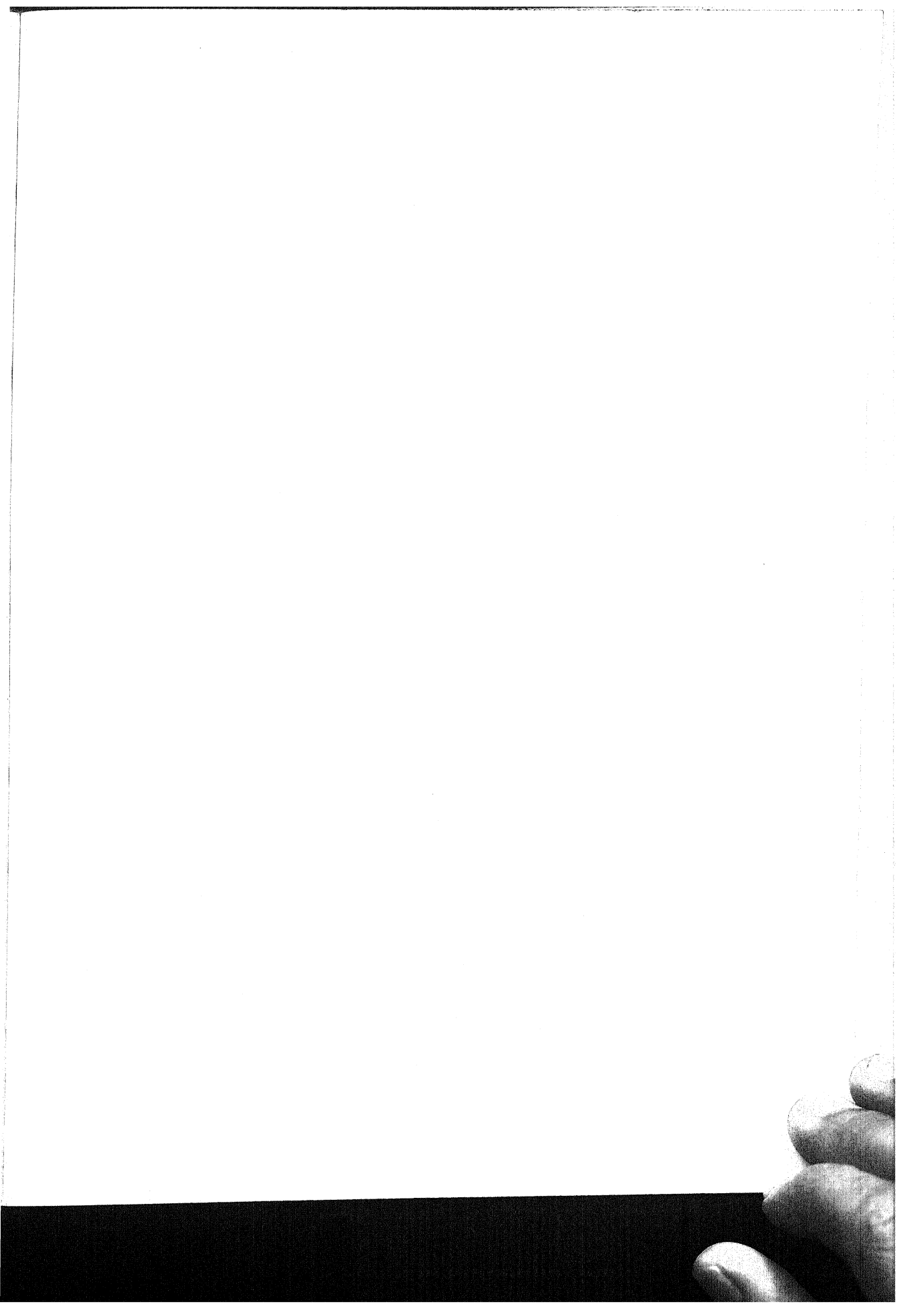

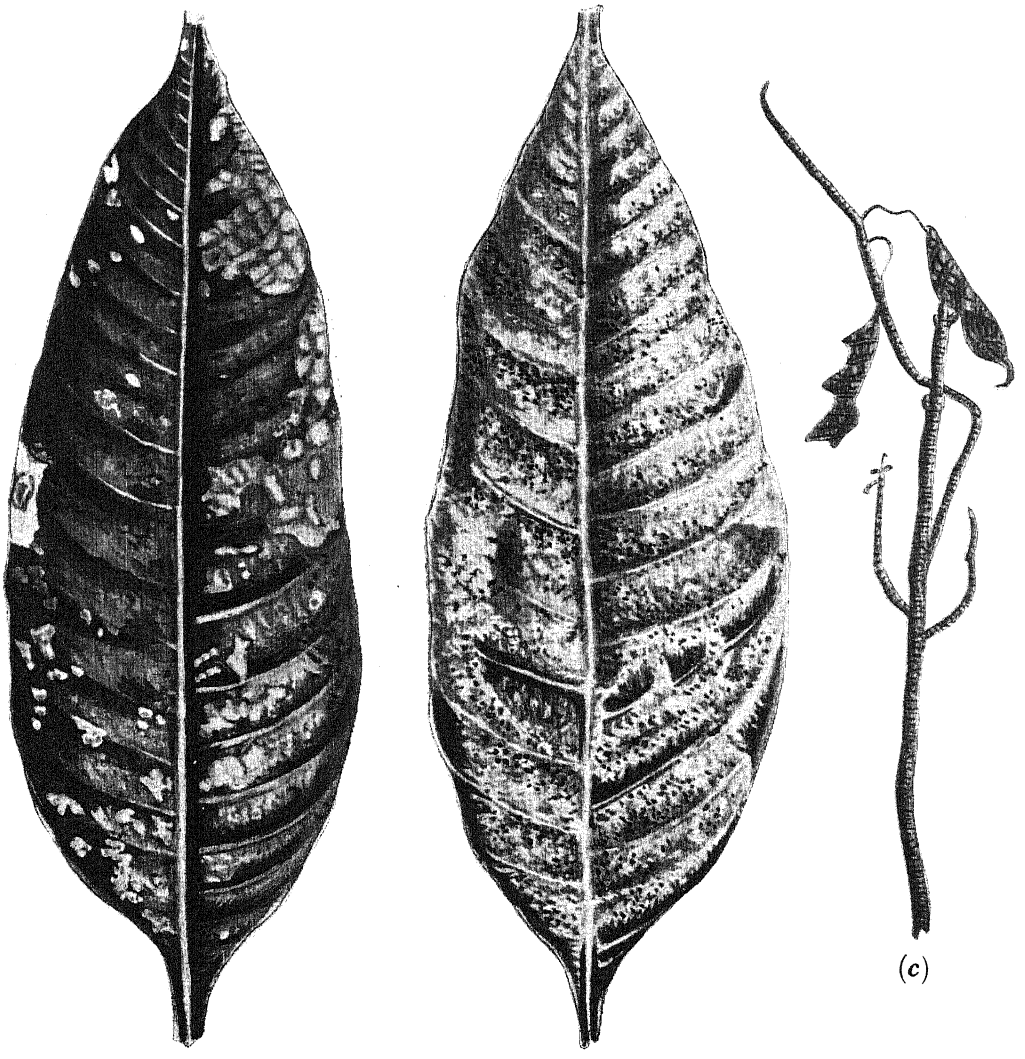

(*a*) (*b*) (*c*)

(*a*) and (*b*) — Mango leaves affected with *Glomerella cingulata* (*Colletotrichum gloeosporioides*)
(*c*) Mango twig affected with *Glomerella cingulata* (*Colletotrichum gloeosporioides*)

SOME STUDIES ON ANTHRACNOSE OF MANGO CAUSED BY *GLOMERELLA CINGULATA* STONEM (S. & V. S.) (*COLLETOTRICHUM GLOEOSPORIOIDES* PENZ) IN THE PUNJAB

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I. Introduction

A report of the occurrence of a disease of mango, which caused drying of leaves and twigs, was received for the first time in February 1934 from a village in the Multan district. After examination of the diseased plants at the spot and in the laboratory, the disease was diagnosed as anthracnose. It was present there in a very serious form. Many young grafted plants, about two to three years old, of varieties imported into that place from other parts of India, were so badly attacked that they ultimately succumbed to the disease.

Later on the disease was found to be prevalent in the districts of Lyallpur, Sheikhupura, Gurdaspur, Jullundur, Ambala and Hoshiarpur. A similar disease of mango due to a species of *Gloeosporium* has been recorded by McRae [1924] from Madras, and due to *Gloeosporium raciborskii* P. Henn. by Uppal, Patel and Kamat [1934] and Stevens and Pierce [1933] from Bombay.

Outside India anthracnose of mango has been recorded in almost all the countries wherever mango is grown. According to McMurran [1914], it was first reported from Porto Rico by Collins in 1903, from Hawaii by Higgins in 1906, from Florida by Fawcett in 1907 and from Cuba by Cardin in 1910. From Uganda it has been reported by Small [1921], from British Guinea by Alston [1925], from Philippines by Ocfemia [1924], from Dominica Republic by Ciferro and Gonyaley Fragaso [1926], from Fiji by Campbell [1927], from Sierra Leone by Deighton [1927], from Guatemala by Popenoe [1919], from Central Andes by Toro [1929] and from South Africa by Doidge [1932].

II. Symptoms of the disease

(*A*) *Macroscopic symptoms*

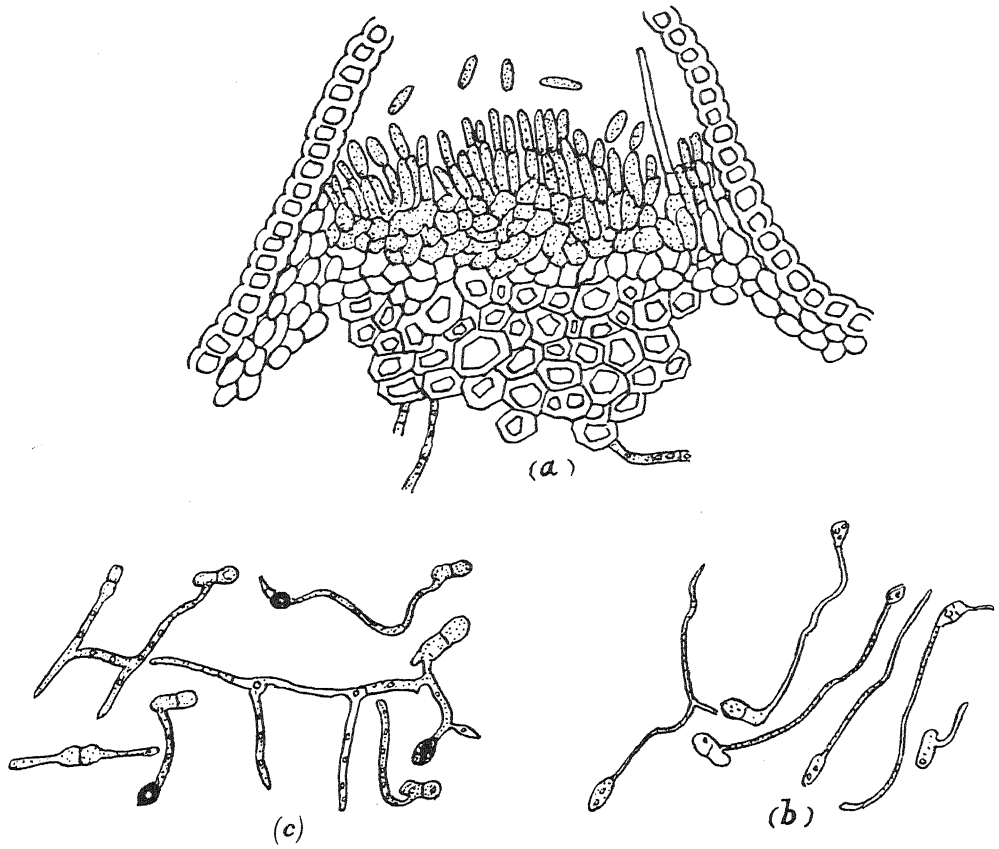
In the Punjab the disease has been observed to attack leaves, petioles, twigs and fruits. On leaves it forms numerous oval or irregular brown spots of very variable size. The spots may begin at the tip or from any other point on the margin or may develop in the centre of the leaf. Under damp conditions the spots grow rapidly, forming elongated necrotic areas which may sometimes result in the rupture of the affected tissue. Young leaves are more severely attacked than old ones.

The petioles, when affected, turn black or grey and the leaves droop down, become dry and ultimately fall off, leaving a black scar on the twig.

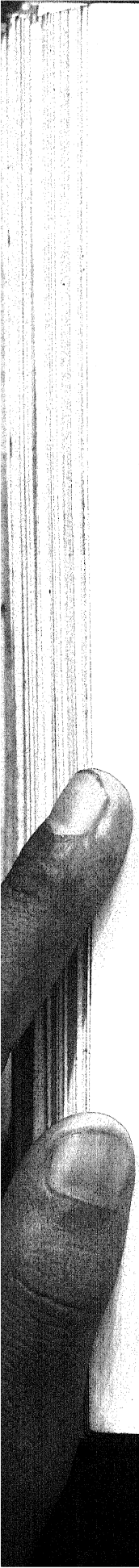
On the twigs the disease produces elongated black necrotic areas. The tips of very young branches are first attacked and the twigs go on drying from top to bottom. Under moist conditions all the branches as well as the main stem of young plants may dry out completely. On older plants young thin branches are also similarly affected, but the big branches are not attacked. When the fruits are attacked, black spots develop on them and rot sets in. The blossom blight has not been observed in the Punjab as yet. Two diseased leaves and a diseased twig are shown in Plate XXI.

(*B*) *Microscopic symptoms*

On diseased parts of the plant the acervuli of the causal fungus (*Colletotrichum gloeosporioides*) develop in great abundance. They are irregular and appear as brown to black dots. On the leaves these occur on both surfaces. The acervuli, when mature exude pink masses of conidia under moist conditions. The acervuli are at first sub-epidermal, but later the epidermis ruptures and exposes them. The acervuli measure 80–250 μ. The marginal setae are rare. When present they are dark, fuliginous, cylindrical, continuous (rarely one-septate), 40-90 × 4-6 μ (mean 60 × 5 μ). The conidia are borne on distinct, well developed hyaline conidiophores. The conidia are straight cylindrical or oval, 8-20 × 5-7 μ, hyaline usually with two, rarely one, oil drops. The hyphae of the fungus as seen in transverse section passing through an acervulus are hyaline, septate, full of oil globules. These are both inter and intra cellular. A transverse section passing through an acervulus is shown in Plate XXII.



(*a*) A transverse section passing through an acervulus of *Glomerella cingulata* (*Colletotrichum gloeosporioides*) (conidial stage); (*b*) Spores of *Glomerella cingulata* (*Colletotrichum gloeosporioides*), germinating in water (conidial stage); (*c*) Spores of *Glomerella cingulata* (*Colletotrichum gloeosporioides*) germinating in $\frac{N}{100}$ malic acid.



III. Pathogenicity of the fungus

The fungus was isolated by the usual methods, from diseased leaves and twigs obtained from Multan, Lyallpur and Gurdaspur. The fungus was purified by monosporal culturing. All the isolations were identical.

The potted plants as well as cut twigs of mango were inoculated by spraying the suspension of spores of the fungus grown on oat-meal agar at 25°C. for eight days. The inoculated plants or twigs were kept moist by keeping them covered under bell-jars or under moist screens of gunny sacks for at least four days after inoculation. The results of inoculation are given in Table I. It is clear that the fungus is pathogenic on leaves, petioles, stem and fruits of mango and that the optimum temperature for infection is about 25°C.

Table I

Results of inoculation experiments

Date of inoculation	Kind of plants inoculated	Temperature, at which inoculated (°C.)	Number inoculated	Number infected	Nature of infection
27th July 1934	Potted seedling plants about 15 months old.	32—36	4	*Nil*	*Nil*
31st July 1934	Ditto .	27—30	4	4	80 per cent leaves attacked.
10th August 1937.	Ditto .	27—30	4	4	80 per cent leaves and 40 per cent petioles infected.
23rd August 1934.	(*i*) Potted seedling plants 15 months old.	27—30	4	4	All leaves and petioles infected.
	(*ii*) Grafted plants 2 years old.	27—30	4	4	70 per cent leaves attacked.
25th September 1934	(*i*) Potted seedling plants 15 months old.	24—28	4	4	Leaves, petioles and stem severely attacked.
	(*ii*) Grafted plants 2 years old.		4	4	80 per cent leaves attacked, a few spots on stem also.
13th August 1934.	Cut twigs from seedling plants, lower ends placed in water.	10	6	*Nil*	*Nil*
13th August 1934.	Ditto .	15	6	*Nil*	*Nil*
13th August 1934.	Ditto .	20	6	6	Infection moderate.
13th August 1934.	Ditto .	25	6	6	Infection very severe.
Do. .	Ditto .	30	6	6	Infection moderate.
Do. .	Ditto .	35	6	*Nil*	*Nil*
Do. .	Ditto .	40	6	*Nil*	*Nil*
14th July 1937	Almost mature mango fruits.	25	12	12	Rotted.

IV. Study of the fungus in artificial cultural media

(*A*) *Macroscopic growth features*

(*i*) *Effect of culture medium.*—The fungus was grown on the following culture media at 20°C. The quantities of nutrients are in one litre solution in distilled water.

Oat-meal agar . . .	Oat-meal 50 grms., and agar agar 20 grms.
Brown's agar . . .	Glucose 2 grms., asparagin 2 grms., K_3PO_4 1·25 grms., $MgSO_4$ 0·75 grms., and agar agar 15 grms.
Brown's starch agar . .	Glucose 2 grms., asparagin 2 grms., K_3PO_4 1·25 grms., $MgSO_4$ 0·75 grms., potato starch 10 grms., and agar agar 15 grms.
Richards' agar . . .	Sucrose 50 grms., KNO_3 10 grms., KH_2PO_4 5 grms., $MgSO_4$ 2·5 grms., and agar agar 20 grms.
Nutrient glucose agar . .	Glucose 20 grms., peptone 10 grms., NaCl 5 grams. extract of meat 4 grms., and agar agar 15 grms.
Potato agar	Peeled potatoes 400 grms., and agar agar 15 grms.

The observations were recorded after twelve days' growth and the various features studied are given in Table II.

Table II

Growth features of the fungus on various culture media

Growth features	Media					
	Oat-meal agar	Brown's agar	Brown's starch agar	Richards' agar	Nutrient glucose agar	Potato agar
Linear colony growth in mm.	88	64	60	103	60	77
Aerial mycelium .	++++	++	++	++++	++++	+
Sporulation . .	++++	+	+++	++++	++	—
Colony colour . .	Salmon buff.	Light salmon orange	Straw-berry pink	Light mineral gray	Flesh pink	Not record-ed

— = *nil*, ++ = moderate, + = trace, +++ = good, ++++ = very good.

(*ii*) *Effect of temperature.*—The fungus was grown on oat-meal and Richards' agars at different temperatures ranging from 5 to 40°C. and the observations were recorded after twelve days. The diameter of colonies of the fungus in both the media at different temperatures is represented in Fig. 1 and the amount of aerial mycelium and sporulation is given in Table III.

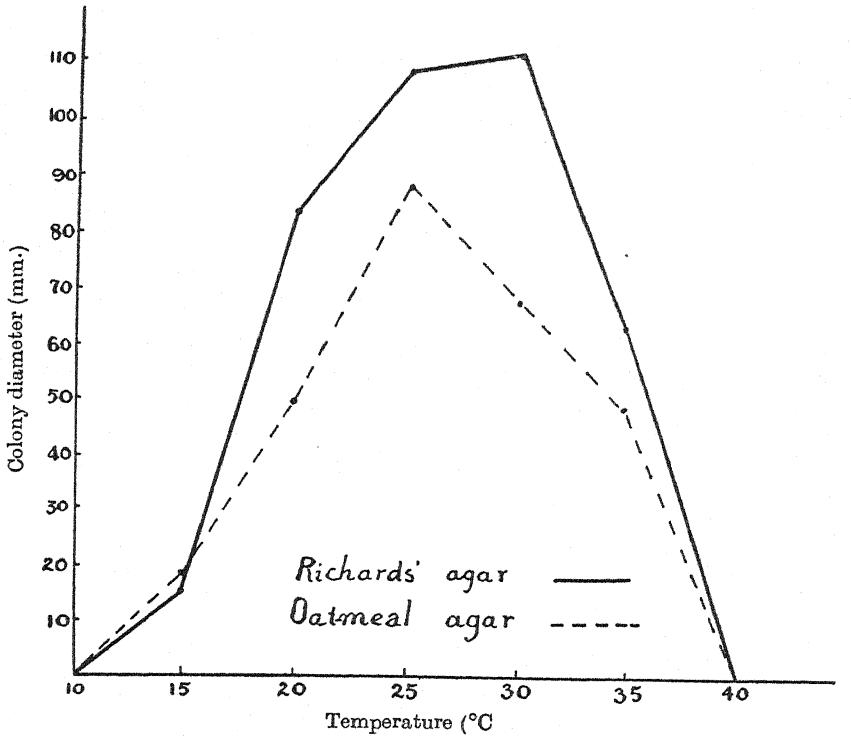


FIG. 1. Effect of temperature upon colony diameter after eleven days

TABLE III

Amount of aerial mycelium and sporulation produced by the fungus at different temperatures

Growth feature	Medium	Temperature (°C.)							
		5	10	15	20	25	30	35	40
Aerial mycelium.	Richards' agar	—	—	+	+++	++++	+++++	++++	—
	Oat-meal agar	—	—	+	++	++	+++	+++++	—
Sporulation	Richards' agar	—	—	—	++	++	++++	+++	—
	Oat-meal agar	—	—	+	+++	++++	+++++	+++	—

— = *nil*, + = trace, ++ = moderate, +++ = good,
++++ = very good, +++++ = abundant.

From Fig. 1, it is clear that the maximum temperature for linear growth of the fungus is between 35 and 40°C. and the minimum is between 10 and

15°C. The optimum temperature is 29°C. for Richards' agar and 25°C. for oat-meal agar, but a close study of the graph reveals that for the culture media in general it seems to be between 25 and 29°C. It is clear from the results given in Table III that the amount of sporulation and aerial mycelium are fairly good on a wide range of temperature, *i.e.*, between 20 and 35°C.

(*iii*) *Effect of hydrogen-ion concentration.*—The medium employed for this study was Richards' agar and the various *p*H values were adjusted colorimetrically by adding normal hydrochloric acid for acid range and sodium hydroxide for basic range. The fungus was grown at 30°C. and observations were recorded after seven days. The linear growth rate at different *p*H ranges is represented in Fig. 2.

The results show that though the greatest linear growth was at *p*H 6·9, the lower ranges up to *p*H 4·2 were all quite suitable for good growth of the fungus. The growth was almost the same from *p*H 4 to 9. At *p*H 3·5 on the acidic side and at *p*H 8·2 on the alkaline side growth fell considerably.

The amount of aerial mycelium was very abundant at *p*H 3·4 to 4·2 and between *p*H 4·2 and 9·0 it was fair, and the amount of sporulation was abundant at *p*H 6·2 to 6·9, fair at *p*H 5·3 and 8·2 and at *p*H below 5·3 and above 8·2 it was scanty or nil.

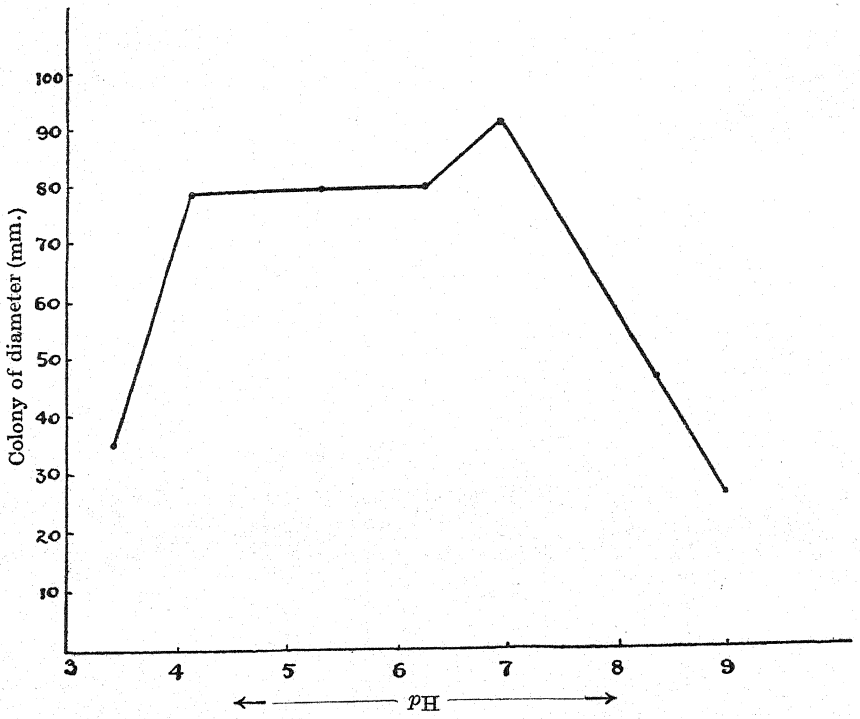


FIG. 2. Effect of *p*H on colony diameter (7 days growth at 20°C. on Richards' agar)

(B) *Microscopic growth features*

Size of spores.—The fungus was grown on five different culture media and the spores were measured after eight days' growth at 20°C. The results are recorded in Table IV. The only point worth noting is that in Brown's agar the spores were the shortest. On all the media the spores were hyaline and continuous.

TABLE IV

Size of spores in different culture media

Culture media	Length in μ		Width in μ	
	Limits of variation	Mean	Limits of variation	Mean
1. Oat-meal agar . . .	8·8—26·0	15·2	3·2—6·8	5·2
2. Brown's agar . . .	8·8—18·0	12·8	3·2—6·0	4·4
3. Brown's starch agar . .	9·2—18·8	14·4	3·2—7·2	5·2
4. Richards' agar . . .	8·8—24·4	13·6	4·0—6·0	4·8
5. Nutrient glucose agar .	8·8—24·8	15·2	3·2—5·6	4·8

The effect of temperature and hydrogen-ion concentration on the size of spores was also studied in some detail but neither of these was found to have any appreciable effect.

(C) *Germination of spores*

(*i*) *Mode of germination.*—The spores of the fungus when sown in drops of distilled water at 20°C. began to produce germ tubes after about six hours and about 7 per cent of the spores became one-septate. Germination went on increasing till after about twenty-four hours it reached a figure of about 70 per cent. Germ tubes generally came out from the ends of spores but occasionally these developed from the middle of the spore length. The germ tubes elongated rapidly and about twelve hours after their formation began to develop appressoria at their ends. The appressoria were knob—or club-shaped structures with a dark thick wall. Often more than one appressorium was formed on a single germ tube. In such cases a hyphal branch was given out from the first formed appressorium and this in turn bore another appressorium.

In *N*/100 malic acid the mode of germination resembled that in water, the only difference being that in this solution about 50 per cent of the spores became one-septate during germination.

(*ii*) *Effect of temperature on germination.*—The germination of spores was studied in water at different temperatures varying from 5-40°C. The spores for the purpose of this study were taken from a fourteen days' old culture of the fungus on oat-meal agar at 20°C. Observations were recorded after twelve, twenty-four and thirty-six hours of sowing and the results are given in Table V.

TABLE V

Germination of spores at different temperatures

Temperature (°C.)	Percentage of germination after			Percentage of of one-septate spores at the end of 36 hours	Percentage of spores which were bearing appressoria at the end of 36 hours
	12 hours	24 hours	36 hours		
5	0	0	0	0	0
10	0	0	0	0	0
15	0	5	9	0	2
20	42	55	63	4	48
25	73	75	85	7	63
30	38	40	45	3	18
35	9	9	11	0	0
40	0	0	0	0	0

From the results given in Table V it is clear that the optimum temperature for germination of spores is 25°C., the maximum between 35 and 40°C. and the minimum between 10 and 15°C.

V. IDENTITY OF THE CAUSAL FUNGUS

Three species of *Gloeosporium*—*Gloeosporium mangae* Noack, *Gloeosporium mangiferae* P. Henn. and *Gloeosporium raciborskii* P. Henn.—have been described on mango [Saccardo, 1902], but from their descriptions it is impossible to separate them. The same view was expressed by Shear and Wood [1913] and the authors also hold the same view. The limits of variation in the size of spores as given in Saccardo are $12\frac{1}{2}$-17 × 3-$3\frac{1}{2}$, 10-16 × 4-$5\frac{1}{2}$ and 12-16 × 4-5 μ for *Gloeosporium mangae*, *Gloeosporium mangiferae* and *Gloeosporium raciborskii* respectively. The limits of variation in the size of spores of the species of *Gloeosporium* described in this paper are 8-20 × 5-7 μ on the host and 8·8-26 × 3·2-7·2 μ in artificial culture media. Thus it is clear that as regards spore size all the three described species of *Gloeosporium* from mango and the one occurring on mango in the Punjab cannot be separated.

Further according to Ocfemia and Agati [1925], Nolla [1926] and Small [1926], the species *Gloeosporium mangiferae* P. Henn. is identical with *Colletotrichum gloeosporioides* Penz. and is considered by Shear and Wood [1913]

and Ocfemia and Agati [1925] to have *Glomerella cingulata* Stonem (S. and VS.) as its perfect stage. The fungus which causes anthracnose of mango in the Punjab should, therefore, be called *Glomerella cingulata* Stonem (S. and VS.) (*Colletotrichum gloeosporioides*).

VI. Modes of perennation of the disease

In order to study the modes of perennation of the disease the following experiments were conducted :—

1. Viability of the fungus in detached diseased twigs and leaves

Twenty diseased twigs and forty leaves affected with anthracnose were removed from mango trees in February 1934 and were kept on the surface of soil in the open at Lyallpur. The viability of the fungus in these twigs was tested from time to time. The results obtained were :—

(*i*) Even after two years' exposure to open weather conditions, the fungus *Glomerella cingulata* (*Colletotrichum gloeosporioides*) could be isolated from the diseased bits of twigs. Seventy per cent of the spores of the fungus borne in acervuli on the twigs were viable. After this period these twigs rotted and were eaten away by white ants.

(*ii*) In the diseased leaves the fungus remained viable for fourteen months. After this period the leaves rotted completely.

As fresh acervuli also developed on diseased twigs and leaves during the period of exposure, it cannot be said whether the viable spores were from the original or fresh acervuli.

(*iii*) Inoculations were made with spores taken from these diseased twigs and leaves on young mango plants. Typical symptoms of the anthracnose disease were produced.

2. Viability of the fungus in diseased twigs persisting on the trees

In nature the infected leaves fall off, but the diseased twigs even after death remain attached to the plant. Twenty diseased twigs on mango trees were marked in February, 1934 and were examined in September 1935, with the following results :—

(*i*) The acervuli were abundant on the marked dead twigs and 80 per cent of the spores in them were viable as proved by germination tests. Fresh acervuli go on appearing on dead twigs persisting on the trees.

(*ii*) Inoculations were made with spores taken from these twigs on young mango plants. These caused the typical anthracnose disease.

Thus from the results of the above experiments it is concluded that so far the disease has been found to perennate by the following methods :—

(1) In the detached diseased twigs and leaves which remain lying on the surface of soil.

(2) In the diseased twigs which may remain attached to the trees.

VII. Methods of control

It is clear from the foregoing account that in order to avoid fresh infection, the sources of infection mentioned therein should be eliminated. The diseased mango leaves, twigs and fruits lying in the garden should be collected and all the diseased twigs on the trees should be pruned away and all such refuse should then be burnt. It is also to be remembered that to achieve good results it is necessary that all the owners of mango gardens in an affected locality should take action simultaneously and co-operatively in their respective gardens. As in practice the total suppression of these sources of infection is not possible, even if these recommendations are followed, the disease cannot be completely controlled, though it can be reduced considerably. Therefore in addition plants have to be sprayed with some fungicide. Trials conducted at Multan on young grafted mango plants showed that spraying the plants with 3 : 3 : 50 Bordeaux mixture three times a year—in February and then in April and September—in addition to the removal of the potential sources of infection above referred to was effective in controlling the disease. Spraying experiments for the control of the disease on plants of bearing age have still to be conducted. In the meantime, however, the following recommendations can be safely made for the control of the disease :—

(1) Remove all the diseased mango leaves and twigs lying about in the garden.

(2) Prune all the diseased twigs and burn them. The cut ends of big branches should be coal-tarred.

(3) Spray the plants with 3 : 3 : 50 Bordeaux mixture three times a year during February, April and September.

VIII. Summary

1. Symptoms of anthracnose disease of mango have been described.

2. The fungus causing the disease has been identified as (*Glomerella cingulata* Stonem (S. and VS.) (*Colletotrichum gloeosporioides* Penz.)

3. Inoculation experiments have shown that the fungus is pathogenic on leaves, petioles, stem and fruits of mango and that the optimum temperature for infection is about 25°C.

4. Effect of different environmental conditions such as nature of medium, acidity of medium and temperature on the growth features of the fungus has been studied.

5. Germination of spores of the fungus has been studied at different temperatures and the mode of germination of spores has been described.

6. The disease has been found to perennate by the following methods :—

(1) In the detached diseased twigs and leaves which remain lying on the surface of soil, and

(2) In the diseased twigs which remain attached to the trees.

7. The following measures for the control of the disease have been suggested :—

(1) Remove all the diseased mango leaves and twigs lying about in the garden.

(2) Prune all the diseased twigs and burn them. The cut ends of big branches should be coal-tarred.

(3) Spray the plants with 3 : 3 : 50 Bordeaux mixture three times a year during February, April and September.

The authors feel indebted to Rai Sahib Jai Chand Luthra, I.A.S., Professor of Botany, Lyallpur, for helpful suggestions and providing facilities during the course of this work. They also feel grateful to M. Ali Mohd., artist for drawing Plate XXI.

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THE PRACTICABILITY OF BIOLOGICAL CONTROL IN THE LAC INDUSTRY

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I. Introduction

The production of lac, the raw product from which shellac, seedlac, and button lac are manufactured is virtually a monopoly in the hands of India. Only approximately 12 per cent of the world's production of stick lac comes from outside India ; of this, roughly 80,000 maunds per annum are produced in Burma, and 60,000 in Indo-China and Siam. The greater part of this *ex*-India production is imported into India and manufactured in this country.

The production of stick lac in India during the last ten years (*i.e.*, 1928-1937) has varied from 9 to 16¼ lakhs of maunds per annum and has averaged 11½ lakhs per annum.

Lac is the resinous secretion of an insect known as the lac insect, *Laccifer lacca* Kerr, which is a member of the Super-Family Coccidae of the Order Hemiptera. This insect derives its nourishment from the sap of certain species of trees and shrubs on which it is parasitic. The majority of these belong to the Leguminosae, and are called lac hosts. There are two varieties of *L. lacca* in the major lac-growing areas, each of which passes through two complete life-cycles during the year, one life-cycle corresponding to a lac crop. These varieties are termed the Rangeeni variety whose two crops are called the *Katki* and *Baisakhi* and the Kusmi variety whose two crops are termed the *Aghani* and *Jethwi*.

Certain lac-growing areas, as for example the Sadar Division of the Palamau District of Bihar, produce almost entirely *Katki* and *Baisakhi* lac and may be termed predominantly Rangeeni areas. The Singhbhum District and parts of the Ranchi District of Bihar, on the other hand, produce mainly *Aghani* and *Jethwi* crops and may be termed predominantly Kusmi areas. Areas in which all four crops are to be found, may be referred to as combined areas.

II. Damage to the lac crops

Damage to the lac crops is of two kinds, both of which occur simultaneously :—

(*a*) Damage due to causes other than insects ;

(*b*) Damage directly due to enemy insects.

(*a*) The former accounts for not less than 30-40 per cent of the original settlement, and is in part unavoidable, as larval settlement is of such density that if mortality did not occur there would be no room for the development of all the cells. It is in part due to climatic conditions, such as intense heat, frost, etc. Part, however, is reducible, being due, for example, to faulty cultivation methods such as incorrect pruning, infection of host trees for crops to which they are not suited, or infection with unsuitable strains of lac.

(*b*) The damage done by insect enemies is approximately 30-40 per cent of the lac cells.

The actual lac crop obtained is approximately 1/4th -1/3rd of the possible hypothetical crop. Of the original settlement 30 per cent is male which takes no practical part in lac secretion. Although only as few as 15-20 per cent of the original settlement may survive to crop maturity, 30-40 per cent, however, takes an appreciable part in lac secretion.

Damage by insect enemies is caused by two main classes of enemies—the parasites and the predators.

III. Damage to the lac crops by parasites

Parasite enemies belong to the Super-Family Chalcidoidea and eight species commonly occur in the major lac-growing areas. These are :—

Encyrtidae—

Parecthrodryinus clavicornis Cam.

Erencyrtus dewitzi Mahd.

Tachardiaephagus tachardiae How.

Tachardiaephagus somervilli Mahd.

Eupelmidae—

Eupelmus tachardiae How.

Aphelinidae—

Coccophagus tschirchii Mahd.

Marietta javensis How.

Eulophidae—

Tetrastichus purpureus Cam.

The damage done by this class of enemy has been shown to be small at Namkum, amounting over seven years to only 4·8 per cent of the lac cells, with an average maximum of only 9·9 per cent.

IV. Damage to the lac crops by predators

A number of predators of the lac insect is known and includes *Ephestia* sp., *Eublemma scitula* Rambr., *Pyroderces falcatella* St., *Chrysopa* sp., etc. Practically the whole of the predator damage to lac, however, is caused exclusively by the larvae of the following :—

1. *Eublemma amabilis* Moore. (Noctuidae)
2. *Holcocera pulverea* Meyr. (Blastobasidae).

The damage by these predators has been shown to amount to 30-35 per cent of the lac cells. It will be seen, therefore, that practically the whole of the insect damage to lac crops is caused by this class of enemy.

Not only is the quantity of lac produced affected by predator enemies, the quality is also lowered by the presence of predator larvae and pupae and their frass. Manufacturing costs are also affected by them because the yield of seed lac on scraped lac is lowered by the presence of the tunnels bored by the predator larvae and by the frass produced by them.

V. Biological control

(*a*) *Biological control of lac parasites*

Three species of hyperparasites of lac parasites have been observed ; in each case, however, the hyperparasite species is in primary function a lac parasite. These species are :

1. *Tetrastichus purpureus* Cam. (Eulophidae.)
2. *Eupelmus tachardiae* How. (Eupelmidae.)
3. *Marietta javensis* How. (Aphelinidae.)

The average percentage hyperparasitism over five years amounted to only 3·6, with an average maximum of only 10·1. In view of this low figure and the fact that the three species are, in primary function, lac parasites, it is clear that biological control of lac parasites is not feasible. In view, however, of the small amount of damage done by lac parasites, this is not of major importance.

(*b*) *Biological control of lac predators*

It is clear, therefore, that biological control in the lac industry actually means biological control of the lac predators, and *E. amabilis* and *H. pulverea* being the two species of major importance as lac predators, it is on the biological control of these species that attention must be concentrated. The following parasites of *E. amabilis* and *H. pulverea* have been bred at Namkum.

1. Parasites of both *E. amabilis* and *H. pulverea*—

Microbracon hebetor Say (Braconidae), larval ecto-parasite, Introduced.

Brachymeria tachardiae Cam. (Chalcididae), pupal endo-parasite.

2. Parasites of *E. amabilis*—

Microbracon greeni Ashm. (Braconidae), larval ecto-parasite.

Aphrastobracon flavipennis Ashm. (Braconidae), larval ecto-parasite. Rare.

Elasmus claripennis Cam. (Elasmidae), larval ecto-parasite.

3. Parasites of *H. pulverea*—

Apanteles tachardiae Cam. (Braconidae), larval endo-parasite.

Pristomerus testaceicollis Cam. (Ichneumonidae), larval endo-parasite.

Eurytoma palidiscapus Cam. (Eurytomidae), pupal endo-parasite.

Periseriola sp. (Bethylidae), larval ecto-parasite.

Elasmus albomaculatus Garh (Elasmidae), larval ecto-parasite. Very rare.

4. Suspected parasites of *H. pulverea*—

Apanteles fakrulhajiae Mahd. (Braconidae), larval endo-parasite.

Chelonella cyclopora Franz. (Braconidae), larval endo-parasite.

Of these parasites of *E. amabilis* and *H. pulverea*, all are indigenous, except *Microbracon hebetor* which is unknown in the major lac-growing areas. Among them, two are of major importance as parasites of lac predators, and can easily be reared in large numbers in the laboratory, these are *Microbracon greeni* Ashm. and *Microbracon hebetor* Say.

Microbracon greeni is an indigenous ecto-parasite of the larva of *E. amabilis*, it can easily be bred in the laboratory, and under field condition is monophagous. The average percentage parasitism throughout the year amounts to approximately 10. This low figure appears to be due to a very great scarcity of host larvae in sufficiently advanced stage for parasitism in the field at certain times of year. As a result of this, the percentage parasitism is very small at such times and the presence of adult parasites in the field very low. When the host density increases, as it does rapidly, the parasite population is still low and it is only some considerable time later that the parasite density increases to a similar extent. Laboratory results indicate that releases of artificially bred adults at such periods would greatly increase the average percentage parasitism.

Microbracon hebetor is an ecto-parasite of both the larvae of *E. amabilis* and of *H. pulverea*. It does not at present occur in the major lac-growing tracts. Adults were obtained from Ceylon by the senior author, where it occurs as a parasite of *Ephestia kühniella* Zell. From these adults a thriving colony has been built up at Namkum. *M. hebetor* can be bred easily in the laboratory and has a number of alternative hosts all of which are pests of

greater or less importance, on which it can breed when host larvae in lac are scarce. Laboratory experiments indicate promising results from the introduction of this Braconid on a large scale in major lac-growing areas. Small-scale field experiments so far carried out show that *M. hebetor* is able to colonise and breed under field conditions.

VI. Proposed large-scale field experiments

The Indian Lac Research Institute has taken over an area situated in the Ranchi District about eleven miles distant from Namkum. This area is predominantly Kusmi, though it contains a small number of hosts producing Rangeeni lac.

Into this area, introductions of *Microbracon greeni* and *Microbracon hebetor* will be made. Regular sampling will be carried out with the object to discover—

(*a*) The effect of periodic releases of *M. greeni* on its population density and on the percentage of hosts parasitised.

(*b*) Whether *M. hebetor* can be artificially introduced and established.

(*c*) The natural population density of *M. hebetor* and percentage of hosts parasitised after establishment.

At the maturity of each crop, a portion will be selected as brood lac (*i.e.* seed for the infection of a new crop), and of this, part will be transferred to another isolated area and used to infect a number of trees for a further crop. Into this second area artificial introductions of these Braconids will not be made. The object being to determine whether—

(*a*) The population density and percentage of hosts parasitised by *M. greeni* can be maintained at a high level by means of adults transferred from one area to another *via* brood lac from an area where periodic liberations of *M. greeni* have been made.

(*b*) Whether *M. hebetor* can be transferred from an area in which liberations have been made to a second area by means of brood lac, and if so, the natural population density and percentage of hosts parasitised in the second area.

It has been thought worth while to place on record the present position as regards these two Braconids, in a predominantly Kusmi area in order to have figures against which to compare the figures from the biological control area chosen.

TABLE I

Density of host population, average percentage parasitism, and density of parasite population, Microbracon greeni *Ashm. per 100 ft. lac in a predominantly Kusmi area, average of seven years (1928-35)*

Month	Presence of *M. greeni* adults per 100 ft. lac	Aghani crop		Jethwi crop	
		Presence of *E. amabilis* larvae in 100 ft. lac	Per cent parasitism	Presence of *E. amabilis* larvae in 100 ft. lac	Per cent parasitism
July	15·4	264 young	0·0	688	7·1
August	6·2	1,704	0·5	Crop cut in June-July re-infected in the following February.	
September	73·4	1,140	8·7		
October	49·0	648	18·2		
November	16·4	744	7·3		
December	11·0	684	15·3		
January	29·0	588	25·4		
February	45·5	336	36·0	0	0·0
March	10·1	252	50·0	168 young	0·0
April	2·1	Crop cut in February-March re-infected the following July.		420 young	0·0
May	1·8			420 young	5·8
June	2·8			288	21·2

(*a*) *Microbracon greeni*

Table I, which is taken from a paper on this species at present in preparation for publication, records the monthly density of *M. greeni* adults and the percentage parasitism in a predominantly Kusmi area, calculated over the seven years, 1928-1935, per 100 ft. of lac in the field. It will be seen that the average number of adults per month per 100 ft. of lac is 22, and the average monthly percentage parasitism 16·3.

The proposed times of liberations of *M. greeni* adults in the field are early August, November and early in May. (This matter is fully discussed in the paper in preparation.)

(*b*) *Microbracon hebetor*

The present density of *M. hebetor* adults in the field and the percentage parasitism are both *nil*.

VII. Recently discovered egg parasites of lac predators

Recently two Trichogrammid egg parasites of the eggs of *E. amabilis*, *H. pulverea* and of a Lycaenid *Tarucus theophrastus* L. have been discovered and have been identified by the Imperial Bureau of Entomology as *Trichogrammatoidea nana* Zehnt and *Trichogramma minutum* Riley. Their potential value in control is great, as they attack their host in the egg stage before any damage has been done. They appear to limit their attack, however, to predator eggs, before the lac encrustation has become thick and continuous and covered with white waxy filaments. In addition, neither *E. amabilis* nor *H. pulverea* lay their eggs in masses. It is at present too early to say anything as regards these two species other than to suggest that their importance in control may be great.

Acknowledgments

We are indebted to Dr. H. K. Sen, Director of the Indian Lac Research Institute, for his interest in this work and to Sir Bryce C. Burt, President of the Indian Lac Cess Committee, Dr. C. F. C. Beeson, Forest Entomologist, Forest Research Institute, Dehra Dun and Dr. H. S. Pruthi, Imperial Entomologist, for criticism and advice.

Summary

1. Insect damage to the lac crops is of two kinds, that due to parasites and that due to predators. The damage done by parasites is small, amounting to only 4·8 per cent ; that due to predators is serious and amounts to 30-40 per cent of the lac cells.

2. Biological control of lac parasites is not feasible ; this is not of major importance as the damage done by them is small.

3. Predator damage is practically entirely caused by the larvae of *Eublemma amabilis* and *Holcocera pulverea*. Biological control in the lac industry amounts to the control of these species.

4. Of the parasites of *E. amabilis* and *H. pulverea* the more important are *Microbracon greeni* and *Microbracon hebetor*. The former is an indigenous parasite of the larva of *E. amabilis*. The latter is an introduced parasite of the larvae of both *E. amabilis* and *H. pulverea*.

5. The average percentage parasitism by *M. greeni* is approximately 10 and the average presence of adults in the field 22 per each 100 ft. of lac. The parasite density becomes very small at certain times of year when hosts are scarce, when the host density increases the parasite density does not increase with the same rapidity. Laboratory tests indicate beneficial results from the release of artificially reared adults at such critical periods.

6. *M. hebetor* does not at present occur in the major lac-growing areas. Laboratory tests indicate that its introduction would be attended by beneficial results.

7. A brief account is given of an experiment that has been started to test the economic value of these Braconids on a field scale.

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TWO NEW CHALCIDOID PARASITES OF THE LINSEED MIDGE, *DASYNEURA LINI* BARNES

I. BIOLOGY AND MORPHOLOGY OF *SYSTASIS DASYNEURAE* MANI

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(With Plate XXIII)

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I. BIOLOGY AND MORPHOLOGY OF *SYSTASIS DASYNEURAE* MANI—

1. Introduction

THE linseed midge, *Dasyneura lini*, was described by Barnes [1936 from material bred from linseed flower buds at Pusa, Bihar, in 1933. A detailed account of its biology has been published by Pruthi and Bhatia [1937] who consider that, in view of the rapid expansion of linseed cultivation in India, this insect is likely to prove an important pest in the future. This forecast is proving to be true, as during the last year reports have been received by the Imperial Entomologist about the seriousness of this pest from several provinces, *e.g.*, the Punjab, Sind, Central Provinces, Delhi, etc.

While this pest was under investigation at Pusa in 1935, occasionally the writer found some ' stumpy ' eggs in the linseed buds, which were quite different from those of the midge, and the larvae hatched therefrom could not be bred on the plant. Similar eggs were also found at Karnal (Punjab) in 1937, which on breeding proved to be those of a Chalcidoid parasite of the midge larvae. The material of *Dasyneura lini*, received recently from Nagpur (Central Provinces) and Sakrand (Sind), has been also found parasitized by the same parasite. The parasite is new to science and is described in part II as a new species under the name *Systasis dasyneurae* by the second author. From the linseed material obtained from Karnal, another chalcidoid also emerged belonging to an undescribed genus, the status of which could not be determined but it is presumably a hyperparasite. This new genus and species are also described in part II. An account of the biology and morphology of the primary parasite, *Systasis dasyneurae*, forms the subject of this part.

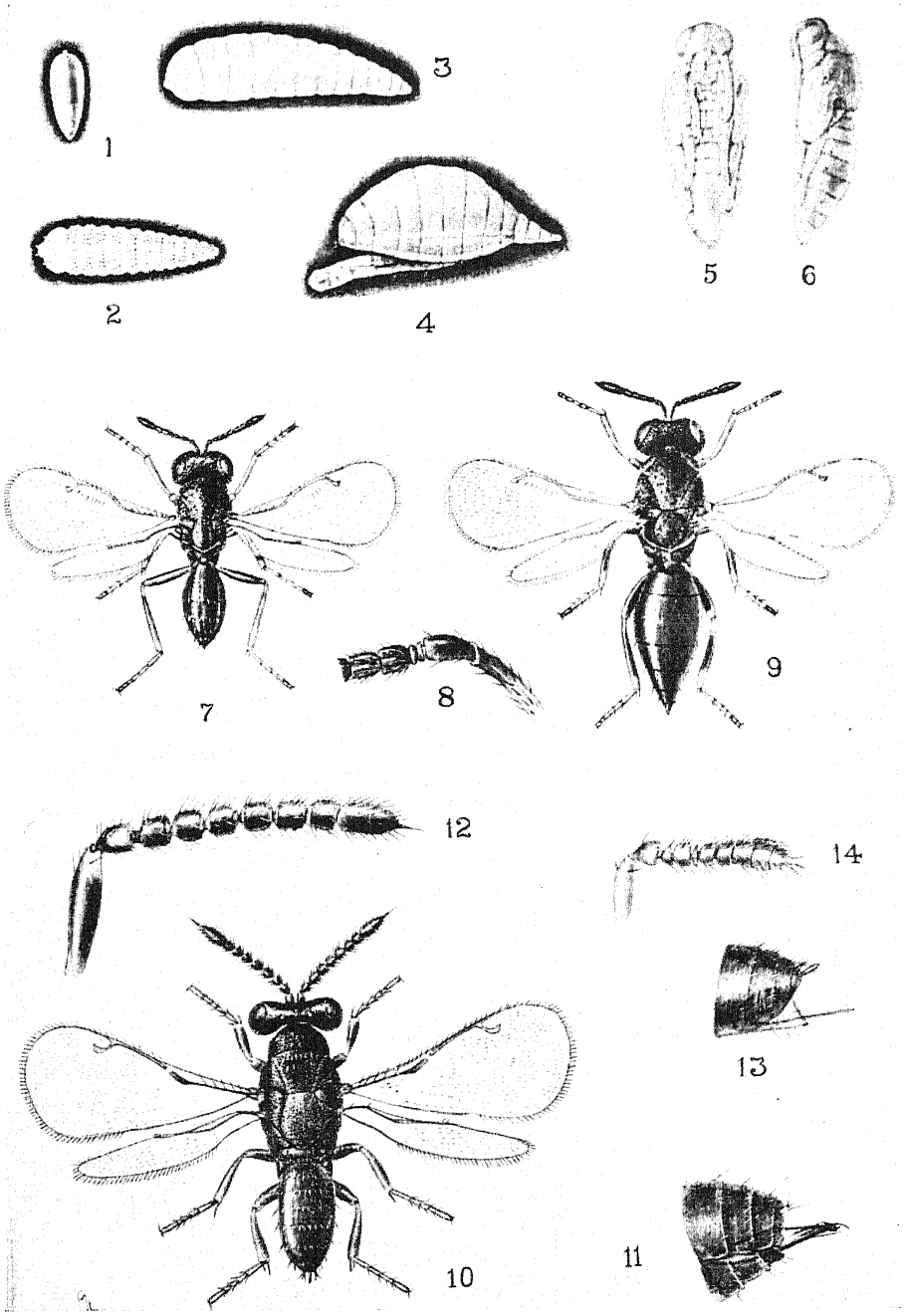
Our sincere thanks are due to Dr. Hem Singh Pruthi, Imperial Entomologist, for facilities for work and valuable suggestions and to Mr. P. Narayanan, the staff artist, who executed the plates with his usual skill under our supervision.

2. Life-history and description of immature stages

Oviposition.—The eggs are laid inside the crumpled and unopened buds of linseed containing well-developed host larvae. The fact that these eggs are invariably found in flower buds already containing almost full-grown larvae of *Dasyneura lini* shows that the parasite possesses not only keen sense of selection but is also capable of distinguishing the affected buds from the healthy ones and further of selecting buds containing host larvae of the right age. Eggs are laid singly but are not necessarily deposited on the host, being more often found on the corolla or stalks and anthers of stamens. They are usually found between the folds of corolla, occasionally on the ovary and anthers, but very rarely outside the corolla in the folds of sepals. Not more than one egg was found in one flower.

Egg.—When freshly laid, the egg is almost transparent, but soon becomes translucent. A little later, a pale yellow band appears all round the egg (Plate XXIII, fig. 1). Just before hatching, the egg becomes darker. It is of a conical or stumpy appearance. At its broad end, the egg is provided with a minute stalk. The surface is marked with microscopic round spots. On an average of twelve readings, the egg measured 0·37 mm. long and 0·13 mm. broad at the thick end.

At a constant temperature of 18°C., the egg stage lasts two to three days. In nature, during February when the nights are rather cool (temperature 15°-18°C.) the incubation period is three to four days. During April (field temperature 25°-29°C.) the eggs hatch in twenty-four to twenty-eight hours.

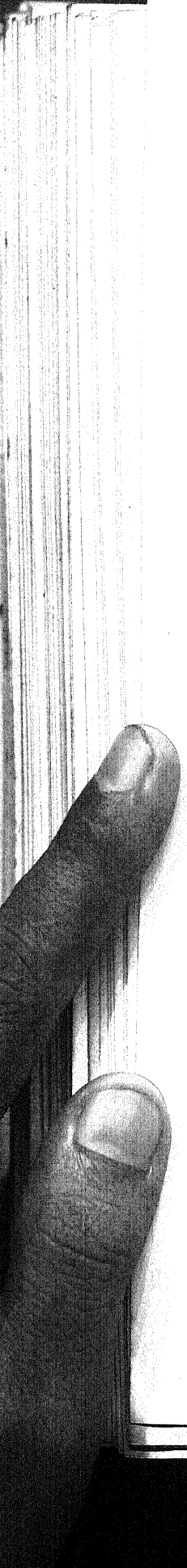


FIGS. 1—9. *Systasis dasyneurae*, sp. nov.

FIG. 1. Egg (×60). FIG. 2. Grub, freshly hatched (×120). FIG. 3. Grub, half developed (×120). FIG. 4. Grub, full developed, on the body-remains of host (×30). FIG. 5. Pupa, ventral view (×30). FIG. 6. Pupa, lateral view (×30). FIG. 7. Adult parasite, male (×35). FIG. 8. Antenna of male parasite, highly magnified. FIG. 9. Adult parasite, female (×35).

FIGS. 10—14. *Ecrizotomorpha taskhiri*, gen. *et* sp. nov.

FIG. 10. Adult, male (×50). FIG. 11. Tip of abdomen of male, highly magnified. FIG. 12. Antenna of male, highly magnified. FIG. 13. Tip of abdomen of female, highly magnified. FIG. 14. Antenna of female, highly magnified.



Grub.—The grub hatches out of the broad end of the egg, where first of all a crescentic slit appears. The young grub may then be seen struggling in the egg for about three quarters of an hour. By this time the head protrudes from the slit and in a few seconds the grub wriggles out completely. The grub immediately after hatching (Plate XXIII, fig. 2) is colourless and almost transparent with the exception of a pale yellow patch (due to the embryonic food inside) extending from the mesothoracic segment to about the fifth abdominal segment. The body consists of the usual three thoracic and ten abdominal segments. It measures about 0·37 to 0·40 mm. long and 0·10 to 0·11 mm. broad. Thus, although the body length of the grub is slightly more than that of the egg, the general shape of the egg is maintained in the grub. The head capsule is large, broadly rounded anteriorly, with strong mandibles in front. The thorax is as broad as, or slightly broader than, the head capsule, the grub having the maximum breadth in the region of the third thoracic segment. The abdomen gradually tapers in the posterior region, the anal segment being narrowly rounded. All the thoracic and abdominal segments are covered with three rows of minute setae, numbering twelve to fourteen per row in the first six segments, but getting fewer as the segments become narrower.

It has been mentioned above that the eggs are almost always laid in buds which already contain well-developed host larvae. Having ensured this, the parasite does not take further care to lay its egg on the body of the host. Therefore the young grub on hatching has sometimes to make a long search for the host. The young grub is extraordinarily hardy and active. It was found capable of wandering about for over twenty-four hours after hatching in search of the host. The terminal portion of its body seems very sensitive and is constantly kept moving as if to serve the purpose of feelers. On coming across a grown-up host larva, it feels it with the last abdominal segment and at once sticks to it. In the initial stages of its growth, the parasite grub makes a minute hole in the body of the host and feeds only on the body juice without injuring its essential organs. At this time its colour is light yellow and the body becomes more elongate, length being about four times the breadth (Plate XXIII, fig. 3, length 0·5 mm., breadth 0·13 mm.). Gradually the thoracic segments increase in size and become conspicuously broader than the head capsule. Later on, the grub begins to feed on the whole body contents of the host leaving only the outer body wall intact. At this stage, it acquires the pinkish orange colouration of the host and its shape also undergoes a considerable change. The body swells up very much in the middle, the first and second abdominal segments marking the maximum width of the larva which is about 0·83 mm. When full grown, the grub measures about 1·8 mm. in length (Plate XXIII, fig. 4). Posterior to the second abdominal segment, the body narrows down almost uniformly, the fifth abdominal segment behind and the head in front having almost equal thickness. The last four abdominal segments remain narrow and continue to move actively and function as feelers.

The first appearance of the swollen pink stage is fairly soon, *viz.*, about four to six days after hatching at 18°C., provided the food supply is abundant. After another four days the grub acquires the maximum size and is full grown. At this time it stops feeding, the pink food inside gets digested and the body colour becomes lighter—first orange yellow, then almost yellow, with a tinge of orange. It contracts, lies motionless for about forty-eight hours and evacuates its excreta which are seen deposited near the posterior end of its body. It finally transforms into a pupa. The total larval period is about twelve to fifteen days (average of ten observations being 13·1 days) at 18°C.

During the entire larval period, the parasite grub consumes three to four host larvae and this number is usually available in one linseed bud. When the parasite egg is laid in buds containing less than three host larvae, it probably gets starved or yields small-sized adults, as it is not capable of moving from one bud to the other. On the other hand, where more than four larvae are found—the maximum so far found is ten—it is probable that all are killed because the parasite has the habit of leaving one host larva partly consumed and passing on to another if available.

Pupa.—The full-grown grub pupates within the bud, the posterior end of the body being glued to the surface of petals. This keeps the pupa secure in the bud and also helps the adult to extricate itself and emerge from the pupal case. The pupa (Plate XXIII, figs. 5 and 6) is naked and measures about 2 mm. in length and 0·75 mm. in breadth.

The pupal period at 18°C. is eleven to fourteen days, average of eleven observations being twelve days.

Pre-imaginal period.—From the foregoing account, it will be noticed that the pre-imaginal period at 18°C. is about twenty-eight days. Large number of linseed buds containing eggs of the parasite were collected from field during February 1937 and the pre-imaginal period was determined at room temperature. Results are shown in Table I.

TABLE I

Pre-imaginal period of Systasis dasyneurae

Date of collection of eggs	Date of emergence of adult parasites	Pre-imaginal period (Days)	Temperature
4th February 1937 . .	3-8th March 1937 . .	27—32	15°-20°C.
5th February 1937 . .	4-10th March 1937 .	27—33	,,
16th February 1937 .	17-20th March 1937 .	29—32	,,

These results confirm the observations carried out at constant temperature of 18°C.

Detailed description of the adult parasite is given in part II.

3. Seasonal history and incidence

At Pusa the midges (*Dasyneura lini*) first appear in the field in the beginning of January and begin to oviposit soon thereafter. The first generation of the midge is completed in two to three weeks ; thus by the end of March, the pest completes about three generations. The incidence of pest is low in January but increases from the middle of February onwards. At Karnal the winters being more severe than at Pusa, the midges become active a little later. The eggs of the parasite on the other hand were abundant in the fields at Karnal from 4th February onwards.

It will be noticed from the account of the life-history given on the previous pages that the parasite is rather slow in its development, requiring about a month to complete one generation in February, but, because unlike other parasites, it is found in large numbers quite early in the season, it gets a comparatively good start over the host.

On 11th February 1937, 3,565 advanced buds of linseed collected at random from a field at Karnal (Punjab) were kept under observation. Out of these, 254 midges, and 87 parasites emerged. At an approximate rate of three midge-larvae killed per parasite the percentage parasitisation works out as follows :—

Number of larvae killed	=261
Number of larvae emerged as flies	=254
Total	=515
Percentage parasitisation	=50·7

This is a fairly high parasitisation for such an early time in the season. It must be however noted that this figure gives the percentage of full-grown larvae parasitised and is not based on the total number of larvae, because buds containing or likely to contain younger larval stages of the midge were not collected. Nevertheless, it is evident that the parasite plays an important part in checking the activity of the pest. Observations taken at Sakrand (Sind) and Nagpur (Central Provinces) seem to confirm this view.

II. Description of the parasites

1. Systasis dasyneurae, sp. nov.

Female.—Length about 1·75-2·0 mm. Bright metallic green. Head transverse, nearly three times as wide as long, somewhat wider than thorax, viewed from in front nearly rounded ; frons convex, bright metallic green, densely reticulately sculptured and rugosely punctured, sparsely and finely setose ; vertex rather narrow mesad, with a depression latero-caudad of the lateral ocelli ; ocelli in a slightly curved line, the front one at apex of the antennal foveae and the lateral ones separated from eye borders by a distance

nearly equal to their diameter ; malar space about threefourths the height of eyes ; clypeus truncate mesad and bidentate laterad anteriorly ; left mandible tridentate and the right with an additional very minute denticle between the second and the third teeth ; eyes dark coppery red. Antennae inserted above an imaginary line drawn from the lower borders of eyes ; segments twelve ; scape reaches the front ocellus, dark above and light brown below ; pedicel very dark metallic green above and somewhat dark brown below ; ring joints 2, first transverse, second thicker and slightly wider ; funicle dark brown, with five subequal segments, the first segment about fourfifths the length of pedicel ; club twice the length of pedicel, triarticulate, first segment about one and one fourth shorter than the fifth funicle segment.

Thorax bright metallic green, closely reticulately sculptured ; mesonotum also with scattered larger, coarser, thimble-punctures ; parapsidal furrows complete, deep ; axillae subreticulately sculptured and slightly advanced into the region of the scapulae ; width of scutellum cephalad is about half the length, sculpture reticulate, without cross furrow before apex. Propodium very short and almost hidden by scutellum mesally, expanding laterally, reticulately sculptured ; with well-developed median and lateral carinae ; median carina forking at apex, the forks running along caudal margin but not specially broad ; lateral carinae at apex running strongly mesad along caudal margin and also laterad to the lateral margin but more faintly ; spiracles large, round, situated near cephalic margin in an obscure impression whose caudal margin is faintly carinate.

Fore wings hyaline, about twice as long as broad, veins dark brown ; marginal vein nearly twice the stigmal vein ; postmarginal vein nearly one and one fourth to twice the stigmal ; stigma round ; submarginal vein distinctly broken before and abruptly more slender than the marginal vein ; there are nine long setae in a straight line caudad of and parallel to the marginal vein ; marginal fringes moderately short. Hind wings not specially narrow, their margins not parallel.

Legs : all coxae and hind femora concolourous with thorax ; basal threefourths of fore and mid femora dark coppery brown ; tibiae and tarsi yellowish brown ; terminal tarsal segment brownish.

Abdomen somewhat longer than the rest of body, conic-ovate or ovate, metallic green or bluish-green ; sculpture scaly, with sparse white pubescence denser apically than basally ; ovipositor not exserted.

Male.—Length about 1·5 mm. Except scape below and at base, antennae dark brown ; funicular segments elongated ; first funicular segment distinctly longer than pedicel but equal to the combined lengths of pedicel and the two ring joints ; second segment nearly equal to the first ; third slightly shorter ; fourth equal to third ; fifth a little over threefourths the length of fourth ; club about as long as the two preceding segments combined ; first segment of club nearly as long as the fifth funicular segment. Mesonotum with the umbilicate punctures more sparse than in the female. Propodium moderately broad mesad. Fore wings with stigma relatively larger ; surface pubescence

somewhat finer. Hind wings relatively narrow with margins subparallel. Fore and mid femora, except basal onethird, dirty brown. Hind femur concolourous with thorax. Abdomen about twothirds the rest of body, other characters as in the female.

Holotype.—One female in spirit, in Pusa collection.

Allotype.—One male in spirit, in Pusa collection.

Paratypes.—Several females and males in spirit and on pins, one female and one male mounted on slides, in Pusa collection.

Host.—*Dasyneura lini* Barnes, second instar larvae.

Coll. T. Ahmad, Karnal (Punjab), Feb.-March, 1937. Also Pusa (Bihar) ; Nagpur (Central Provinces).

This new species resembles *Systasis varipes* Girault [1915] in size, colour, and in the lateral carinae on propodium but differs in the relatively longer postmarginal vein. From *S. insularis* Dodd & Gir. [1915], which it approaches more closely, it is readily distinguished by the differently coloured fore and mid legs and longer postmarginal vein. From *S. henrici* Gir. [1915] it differs in the tridentate mandible.

The genus *Systasis* Walk. [1834], recorded here for the first time from India, comprises fifteen species, cosmopolitan, but mostly described from Australia so far. It belongs to the tribe Tridymariae of the family Miscogasteridae and is easily recognised by twelve-jointed antennae (with two ring-joints) inserted in the middle of face, reticulately sculptured thorax with scattered umbilicate punctures, hyaline wings, marginal vein being twice the stigmal, and by the row of long setae behind marginal vein.

2. *Ecrizotomorpha*, gen. nov.

This new genus is erected for a Pirenine bred from linseed midge along with *Systasis dasyneurae*. It is readily recognised by the pubescent eye, 10-jointed antennae, triarticulate maxillary palpi, long stigmal and postmarginal veins, stout and compressed posterior tibiae and normal second abdominal tergite. From *Herbertia* Howard [1894], which it resembles in the eye pubescence and number of antennal segments, it is easily distinguished by the well-developed stigmal vein and normal second abdominal tergite. From *Ecrizotes* Forster [1861], which it approaches most closely in venation and moniliform antennae of male, it is separated by the pubescent eyes, absence of ring-joints and triarticulate maxillary palpi. It also bears a superficial resemblance to *Spathopus* Ashm. [1904] in its stout and compressed hind tibiae and short stouter fore tibiae but differs in the longer hind tibiae, pubescent eyes, absence of ring-joints and clypeus produced obtusely but not triangularly. It is not likely to be confused with the other genera of the subfamily. In Ferriere's [1934] key this genus runs to couplet number 13, but differs in the funicle having only the first and third segments transverse in female and by the prominent hypopygium and ovipositor.

The following is a full description of the genus :

Non-metallic. Head transverse, thin mesad, narrow at vertex, wider than thorax, rounded in front view. Eyes large, pubescent, not converging above. Clypeus obtusely produced anteriorly. Maxillary palpi triarticulate. Mandibles tetradentate. Antennae short and clavate in female, inserted low down in the face in level with the lower border of eyes ; segments ten; ring-joints absent ; scape long, broad, sub-compressed ; pedicel large, short ; first and third funicular segments transverse and resemble ring-joints ; club triarticulate, terminal segment armed with three spines apically ; in male funicle moniliform, closely pilose, club biarticulate, terminal segment ending in a nipple-like prolongation bearing a long spine. Thorax flat, depressed, finely scaly and with a few scattered setigerous, minute punctures. Fore tibiae stouter and shorter than femora ; in male hind tibiae longer than femora, broad, compressed apically. Wings hyaline ; submarginal in fore wings abruptly slender and broken before uniting with marginal vein ; marginal vein twothirds the submarginal, also twice the well-developed stigmal vein ; postmarginal vein somewhat shorter than stigmal. Parapsidal furrows well developed and deep. Propodium not short mesad, smooth, ecarinate, not produced at apex ; spiracles round and cephalad ; spiracular sulci present. Abdomen in female equal to or somewhat longer than the rest of body, ovate, sessile, second tergite short ; ovipositor and its sheath exerted but not much ; in male variable, compressed and oval or elongate oval and normal, shortly petiolate, second tergite as in female ; hypopygium prominent.

Genotype.—E. taskhiri, sp. nov.

3. Ecrizotomorpha taskhiri, sp. nov.

Female.—Length varies between 0·9-1·5 mm., the average being about 1·25 mm. Black, non-metallic. Head slightly wider than long, thin at vertex and thick near mouth, with a few scattered setigerous punctures ; clypeus slightly metallic blue. Ocelli almost in a line. Scape dark reddish brown, a little over half the length of flagellum ; rest of antenna reddish brown. Pedicel much shorter than club. First funicular segment short, transverse, narrow, trapezoid, resembling a ring joint ; second funicular segment subquadrate, twice the length of first, broader ; third segment like the first ; fourth and fifth subequal to second but wider ; club threefourths the length of scape, much wider than last funicular segment, segments equal ; terminal segment obtusely reduced and broadly rounded apically, with two long, stout, lateral spines and a much longer, slender median spine. Thorax black, scaly, with few scattered punctures or none, but setose. Veins dark brown. Except coxae and femora which are concolourous with thorax, legs light brown ; tibiae slightly darker outside basally ; knee pale.

Male.—Length varies between 1·0-1·5 mm. Black. Pedicel somewhat longer than the first funicular segment. Funicular segments subglobose, except last which is somewhat elongated. First segment of club half the length of club and one and onefourth the last funicular segment. Hind tibiae dark brown. Otherwise as in female.

Holotype.—One female in spirit, in Pusa collection.

Allotype.—One male in spirit, in Pusa Collection.

Paratypes.—Several females and males in spirit and on cards, two females and males mounted on slides ; in Pusa Collection.

Coll. T. Ahmad, Karnal (Punjab), Feb.-March, 1937. Status unknown ; possibly a hyperparasite of *Systasis dasyneurae.*

Summary

Part I deals with the biology and morphology of *Systasis dasyneurae*, a larval parasite of the linseed midge, *Dasyneura lini*. Eggs are laid singly inside unopened flower buds of linseed containing well-developed larvae of the midge, revealing thereby a marked sense of selection on the part of the parasite. The young grub on hatching is fairly hardy and can roam about for over twentyfour hours in search of host larvae. The last four abdominal segments of the grub are very narrow as compared to thorax and the remaining portion of abdomen, and are kept actively moving about to search and feel the host larvae. Before pupation, the grub consumes three to four larvae and this number is usually available in one linseed bud. The full-grown grub pupates within the bud with its posterior end glued to the surface of petals. At 18°C. the egg stage lasts two to three days, the grub stage twelve to fifteen days and the pupal stage eleven to fourteen days. Thus under these conditions one generation is completed in a little less than a month. The parasite seems to play an important part in controlling the pest, as unlike many other parasites it is fairly abundant early in the season. In February 1937 as many as 50·7 per cent of full-grown larvae in buds collected from Karnal (Punjab) were estimated to have been killed by the parasite.

Part II gives the descriptions of *Systasis dasyneurae*, sp. nov. and another Chalcid *Ecrizotomorpha taskhiri*, gen. *et* sp. nov. The exact status of the latter is not known but presumably it is a hyperparasite on the former.

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STUDIES ON THE COCONUT PALM BEETLE (*ORYCTES RHINOCEROS* LINN.) IN SOUTH INDIA

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(With Plate XXIV)

INTRODUCTION

COCONUT palms in South India are subject to infestation by at least three major pests, *viz.*, the coconut palm beetle (*Oryctes rhinoceros*), the palm weevil (*Rhynchophorous ferrugineus*) and the black-headed caterpillar (*Nephantis serinopa*). Of these, the beetle is most widespread and persistent in all coconut-growing centres in the Madras Presidency and the adjacent states of Cochin, Travancore and Mysore. It is reported to be a serious pest also from other provinces, such as Bombay and Bengal. Outside India, it has been recorded from several countries, such as Burma, Ceylon, Singapore, Sumatra, Java, Celebes, the Philippine Islands, etc.

Several authors have made observations on this insect, *viz.*, Fletcher [1914] and Ramchandra Rao [1930] in South India, Ghosh [1911] in Burma, Hutson [1933] in Ceylon and Mackie [1917] in the Philippine Islands. The beetle has been reported upon also, in the report of the Proceedings of the Second Entomological Meeting, Pusa, by various workers in the different provinces. From a perusal of the literature, it appears that there are variations in the life-history, depending with locality and season where the pest was studied and therefore it is necessary that more data should be collected by further intensive study. During the past three years some interesting observations have been made in the field and the laboratory at Coimbatore and the results thereof are presented hereunder.

HABITAT

While the adult beetle spends part or whole of its life in the crowns of trees (coconut, palmyra or Talipot palms), at no other stage it is found in the living tree. The eggs are invariably laid in the heaps of farmyard manure. Occasionally the beetle, eggs and larvae are also found in decaying vegetable matter, such as dead coconut trunks and stumps, decomposing damp choir refuse in coastal tracts, heaps of wet sugarcane trash, and almost any kind of compost in fermenting condition. Invariably, a tree destroyed by the palm weevil disintegrates and becomes easily a veritable breeding ground

for the beetle larvae. But more commonly, the manure pits of dung and trash, when kept undisturbed, serve as a favourite breeding place for the beetle in all the tracts in South India.

Life-cycle and description of immature stages

Egg.—The female beetle burrows into the partly dried-up dung heap and lays its eggs and, under such situations, it is mostly unaccompanied by its male partner. The eggs are laid two to six inches below the surface in semi-wet dung, each egg being deposited individually and covered over with dung material. The egg is almost oval or elliptical in form, measuring 3·1 ×2·1 mm. on an average. Before hatching, the creamy white colour changes to yellowish brown and the average size of the now almost spherical egg comes to 4·0×3·4 mm. The egg period varies a good deal taking seven to twenty days as will be seen from the rearing record given below. It is also known from rearings at the Insectary that the eggs laid on the same day, do not all hatch within the same interval and that a number of eggs in each lot fail to hatch due to natural causes.

Table I

*Observations on the duration of the egg period**

Month	Number of days			Remarks
	Minimum	Maximum	Average	
1936				
May	7	14	9·24	25 eggs examined under field conditions.
June	8	14	10·00	18 eggs examined under field conditions.
July	13	16	14·50	21 eggs examined under field conditions.
August	9	11	9·75	12 eggs examined under field conditions.
September	7	18	12·81	37 eggs examined under field conditions.
October	15	20	17·36	11 eggs under laboratory conditions.
November	8	11	8·88	11 eggs under field conditions.
December	9	15	11·05	17 eggs under laboratory conditions.
1937				
January	13	13	13·00	6 eggs under laboratory conditions.
February	11	14	12·60	15 eggs under field conditions.
March	9	14	10·43	32 eggs under laboratory conditions.
April	9	13	11·20	20 eggs under field conditions.

* The exact egg period was ascertained by examining the cages daily.

Grub and its habits.—The newly hatched grub measures 5·5 to 6·0 mm. in length and is whitish in colour with a pale brown head. It commences feeding soon after hatching and, in the course of about forty days, attains a size of about 100 mm. by active feeding and growth. Afterwards, there is practically little change in size. The full-grown grub is clumsily heavy and sluggish in its movements and it always seeks refuge deep below the surface layers of breeding material. The grubs are found at a depth of 2-12 inches, in the semi-wet dung cakes. During unusually dry period, with scanty dung material, young grubs perish and older ones attempt to bury themselves in the deeper layers of moist earth. During periods of active feeding, large quantity of dung material and decomposing vegetable matter are ingested and thrown out as conspicuous pellets and castings. The mature grub when handled exudes large quantities of liquid through its mouth, and semi-solid black matter through its anal opening. The grub, preparatory to pupation, spends a good deal of time in probing the substratum to locate a suitable place for cell formation. Invariably, the earthern cell is excavated in the sides of the pit, usually 6-12 inches below surface level or rarely lower down in the bed of the pit where sufficient moisture is available. The grub, by its wriggling movements, smoothens the walls of the oval chamber now formed and the liquid and the faecal matter exuded in small quantities dry up to make a black smooth lining to the cell or chamber. When completed, the ball-like mass of earth or dung shows no opening or hole on the surface. The grub inside remains as such for about a week or more as could be observed from the cells constructed close to the glass sides of the cages under laboratory rearing. The grub inside the cell gradually shrinks in size and the larval skin by its last moult is thrown off in the cell itself. Some grubs do perish in their efforts at cell formation and pupation.

Under natural conditions, the grubs appear to desert the dung long before pupation and burrow into the soil close to the feeding material. In the course of rearing in the Insectary, a few grown-up grubs were placed in a special wooden cage, which was divided into two compartments by a vertical wire-gauze frame across, leaving enough space at the bottom for the passage of the grubs from one chamber to the other. One compartment was filled with earth and the other with dung cakes, and both kept semi-wet. The grubs were all let into the dung chamber, and at the time of pupation it was found that all slowly found their way to the earthern chamber and pupated there, while not one of them pupated in the dung. It is also noted that, in many cases, a number of grubs perish at the time of pupation, if dung only is supplied. In successful cases, with the dung for pupation, the grub

stage was found protracted and by the time pupation takes place, the dung by feeding and drying is almost reduced to the condition of powdery earth. In field pits, normally the grubs move away from the breeding material to the sides and bottom of the pit, and bury themselves in the earth for pupation. In some cases white ants are seen invading the manure pits, and by their activities an admixture of earth with dung is brought about, and a few of the grubs have been found to pupate in this powdery mixture of earth and dung. The presence of wet soil nearby appears to be a favourable condition for successful pupation. The larval period is pretty long and it varies for different lots or for different specimens of the same lot, both under field and laboratory conditions. The wide variations in the larval period, noticed in the course of a few rearings, are recorded in Table II.

TABLE II

Duration of larval period

Month	Minimum in days	Maximum in days	Average in days	Remarks
1936				
May	124	152	133·6	3 rearings
June	98	136	115·3	6 ,,
July	87	102	99·5	2 ,,
September . . .	93	168	134·0	4 ,,
October . . .	182	182	182·0	1 ,,
November . . .	133	150	138·7	4 ,,
December . . .	103	159	126·4	7 ,,
1937				
February . . .	130	139	135·6	3 ,,
March	88	130	116·0	3 ,,

Pupa.—The newly formed pupa is pale brown and partly covered by the larval exuvium. Later, it becomes reddish brown showing the rudiments of the appendages of the adult folded within the pupal skin. In later stages, the pupa acquires a darker brown tint. The pupal skin is thrown off in the cell, and the adult beetle remains inside the cell for over ten days before it

comes out or shows activity. The pupal period, from cocooning to the emergence of the adult from the cell, varies from seventeen to sixty-two days as observed in the following rearings.

TABLE III

Duration of pupal period

Month	Minimum in days	Maximum in days	Average in days	Remarks
1936				
May	17	32	24·6	6 rearings in lab.
	29	29	29·0	1 rearing in field.
June	28	28	28·0	2 rearings in field.
September	31	31	31·0	1 rearing in lab.
	62	62	62·0	1 rearing in field.
October	34	38	36·0	2 rearings in lab.
	21	35	28·5	9 rearings in field.
November	43	43	43·0	1 rearing in field.
December	31	31	31·0	1 rearing in lab.
	25	54	44·4	3 rearings in field.
1937				
January	28	28	28·0	4 rearings in lab.
February	35	35	35·0	1 rearing in lab.

In view of the fact that the period from cocooning to emergence includes also stages of inactive larva as well as inactive adult within the cell, wide variations are inevitable. In a few instances, these different periods inside the cell have been observed by keeping some individuals close to the glass walls of the cage or by making a small opening in cell for examination. Such details of observations are given in Table IV.

TABLE IV

Cell formation	Pupation	Becoming adult	Emergence out of the cell	Actual period as pupa (days)
16th Sept. 1936	26th Sept. 1936	16th Oct. 1936	28th Oct. 1936	20
16th ,,	28th ,,	18th ,,	28th ,,	20
,,	28th ,,	18th ,,	27th ,,	20
,,	28th ,,	22nd ,,	27th ,,	24
5th Oct. 1936	16th Oct. 1936	9th Nov. 1936	18th Nov. 1936	24
5th ,,	16th ,,	9th ,,	16th ,,	24
31st May 1937	12th June 1937	22nd June 1937	30th June 1937	11

If, during early stage of cell formation, the grub is disturbed, grubs have been known to rebuild fresh cells twice and thrice.

Total life-cycle

The total life-cycle in a few typical cases of rearing from egg to adult is given in Table V.

Table V

Duration from egg to adult

Egg laid on	Adult emerged on	Life-cycle in days	Remarks
21st Dec. 1935.	1st June 1936	162	Reared in the laboratory.
21st ,, ,,	29th May ,,	159	
17th Sept. 1936	17th ,, 1937	232	
15th Dec. ,,	27th ,, ,,	163	
15th ,, ,,	12th June ,,	179	
2nd Nov. ,,	17th May ,,	196	
30th Jan. 1937.	28th July ,,	179	
10th Feb. ,,	28th ,, ,,	168	
21st ,, ,,	30th June ,,	129	
20th May 1936.	22nd Oct. 1936	155	Reared in the manure pits.
22nd ,, ,,	6th Nov. ,,	168	
23rd ,, ,,	23rd ,, ,,	184	
7th June ,,	6th ,, ,,	152	
8th ,, ,,	6th ,, ,,	151	
15th July ,,	30th Dec. ,,	168	
13th ,, ,,	21st Jan. 1937	192	
20th Aug. ,,	21st ,, ,,	154	
4th Sept. ,,	23rd ,, ,,	141	

The temperature and humidity at which the life-history of the beetle was studied are given in Appendix I.

Egg-laying records

Few workers have indicated large scale trials on the egg-laying capacity of the beetle. The paucity of records on the egg-laying habits of the beetle appears to be due to the fact, that many specimens kept in captivity die away without laying eggs. Ghosh [1911] records " several pairs were placed separately but only two females laid eggs ", one laying eight eggs and the other four eggs. Mackie [1917] has indicated only a probable maximum of thirty eggs. At Coimbatore, under laboratory conditions, large number of beetles were reared out and induced to lay eggs in captivity, providing a variety of conditions. Considerable difficulty was experienced, in the initial stages, in giving a proper feed to the beetles in cages. In the course of the trials, it was found, that the beetles in captivity could be fed more successfully with cane stems than anything else. Out of twenty-four attempts, egg-laying was observed in a dozen cases, and the records relating to a few typical cases are given in Table VI. Under field conditions, a fertilised female, that has entered a suitable manure pit for laying eggs, is observed to remain there for a number of days laying a few eggs each day or at intervals. A few observations on the beetle's egg-laying in field pits are shown in Table VII. It will be seen from Table VI that a single female beetle laid as many as 140 eggs which is the maximum number recorded and two beetles collected from the field and kept in captivity in field cages in manure pits laid over 100 eggs each.

Longevity records

The duration of life of the adult is also subject to great variation under varying circumstances. The study of longevity is particularly important in this instance, in view of the direct injury to the palms caused by the adult beetles. The past records on the longevity of the beetle are scanty. Mackie [1917] puts the limit as eighty-five days and other workers do not seem to have paid much attention to the details of this aspect. In the course of rearings in the Insectary at Coimbatore, during the past three years, the longevity was studied in a number of cases and the longevity records with reference to twenty-eight typical cases are shown in Table VIII. It will be seen from the table that (1) one female beetle lived for 293 days, (2) one male lived for 231 days, and (3) in seven other trials the specimens lived for more than 200 days.

TABLE

Egg-laying records under

Serial No.	Date of emergence of pair	Preoviposition period in days	1st batch		2nd batch		3rd batch		4th batch		5th batch		6th batch		7th batch	
			Date of egg-laying	No. of eggs	Date of egg-laying	No. of eggs	Date of egg-laying	No. of eggs	Date of egg-laying	No. of eggs	Date of egg-laying	No. of eggs	Date of egg-laying	No. of eggs	Date of egg-laying	No. of eggs
1	20-9-36 ♂ 21-9-36 ♀	142	10-2-37	7	11-2-37	1	16-3-37	11	17-3-37	1	19-3-37	5	21-3-37	10	25-3-37	16
2	21-9-36 ♂ 20-9-36 ♀	92	21-12-36	12	13-2-37	3	21-3-37	14	25-3-37	12	29-4-37	4	5-6-37	16	7-6-37	7
3	20-9-36 ♂ 16-10-36 ♀	17	1-11-36	17	2-11-36	2										
4	28-10-36 ♂ 28-10-36 ♀	39	5-12-36	3	8-12-36	3										
5	28-10-36 ♂ 28-10-36 ♀	38	4-12-36	2	10-1-37	24	31-1-37	5	16-2-37	6	28-2-37	5	1-3-37	5	3-3-37	12
6	28-10-36 ♂ 5-2-37 ♀	23	28-2-37	10	8-3-37	2	10-3-37	14	12-3-37	14	26-27-3-37	53	29-3-37	8	2-5-37	12
7	17-5-37 ♂ 7-5-37 ♀	25	12-6-37	9	4-7-37	4	10-7-37	13	18-7-37	6	13-8-37	30	16-8-37	19	22-8-37	6
8	17-5-37 ♂ 12-6-37 ♀	29	11-7-37	9	23-7-37	6	15-16-8-37	39								

VI

laboratory conditions

8th batch		9th batch		10th batch		11th batch		12th batch		13th batch		14th batch		Date of death	Total No. of eggs
Date of egg-laying	No. of eggs	Date of egg-laying	No. of eggs	Date of egg-laying	No. of eggs	Date of egg-laying	No. of eggs	Date of egg-laying	No. of eggs	Date of egg-laying	No. of eggs	Date of egg-laying	No. of eggs		
31-3-37	16	3-4-37	9	7-4-37	24	8-4-37	18	9-4-37	4	10-4-37	14	13-4-37	4	26-4-37 ♂ 24-4-37 ♀	140
9-6-37	2	21-6-37	6	27-6-37	15	30-6-37	8							10-5-37 ♂ 10-7-37 ♀	99
														21-12-36 ♂ escaped. 25-1-37 ♀	19
														25-3-37 ♂ 19-3-37 ♀	6
12-3-37	1	24-3-37	31	25-3-37	49	10-5-37	40	13-5-37	25	15-5-37	15	31-5-37	3	15-5-37 ♂ 31-5-37 ♀ 223 11-6-37 ♀ 2	
3-4-5-37	32	7-8-5-37	53	12-5-37	30	13-5-37	16							18-3-37 ♂ 17-4-37 ♂ 13-5-37 ♀ 244 12-6-37 ♀ 2	
														13-9-37 ♂ 1-9-37 ♀	87
														12-9-37 ♂ 7-9-37 ♀	54

TABLE

Egg-laying records

Serial No.	Date of capture of female	Eggs at the time of capture.	1st batch		2nd batch		3rd batch		4th batch		5th batch	
			Date of egg-laying	No. of eggs	Date of egg-laying	No. of eggs	Date of egg-laying	No. of eggs	Date of egg-laying	No. of eggs	Date of egg-laying	No. of eggs
1	20-5-36 .	36	21-5-36	7	22-5-36	6	23-5-36	6	24-5-36	4	25-5-36	5
2	4-6-36 . .	69	5-6-36	1	7-6-36	9	8-6-36	4	9-6-36	2	11-6-36	4
3	9-7-36 . .	*Nil*	13-7-36	17	14-7-36	5	15-7-36	2				
4	26-8-36 .	24	28-8-36	20	29-8-36	2	4-9-36	45	13-9-36	1	16-9-36	4
5	2-9-36 . .	74	27-9-36	8	1-10-36	3	4-10-36	5	7-10-36	9	11-10-36	4
6	30-10-36 .	*Nil*	15-11-36	1	18-11-36	12	25-11-36	1	26-11-36	1		
7	10-2-37 .	*Nil*	18-19-2-37	10	21-2-37	13						
8	8-4-37 . .	44	14-4-37	17	15-4-37	7	16-4-37	12	25-4-37	2		
9	14-5-37 .	*Nil*	29-5-37	2	21-22-6-37	6						

VII

in manure pits

6th batch		7th batch		8th batch		9th batch		10th batch		Date of death	Total No. of eggs
Date of egg-laying	No. of eggs	Date of egg-laying	No. of eggs	Date of egg-laying	No. of eggs	Date of egg-laying	No. of eggs	Date of egg-laying	No. of eggs		
26-5-36	5	29-5-36	1	30-5-36	2	31-5-36	1	1-6-36	1	4-5-36	94
13-6-36	3									17-6-36	92
										28-7-36	24
17-9-36	8	18-9-36	3	22-9-36	7	23-9-36	1			5-10-36	115
18-11-36	12									27-11-36	115
										29-11-36	15
										3-3-37	23
										10-5-37	82
										24-6-37	8

TABLE VIII

Longevity records under laboratory rearing

Serial No.	Date of emergence	Date of death	Longevity in days		Remarks
1	9-8-35	25-8-35	Female	16	
2	9-8-35	5-9-35	Male	27	
3	27-3-36	17-4-36	Female	21	
4	27-3-36	20-4-36	,,	24	Reared specimens—kept without feed.
5	27-3-36	22-4-36	,,	26	
6	27-3-36	22-4-36	,,	26	
7	27-3-36	22-4-36	Male	26	
8	27-3-36	4-5-36	,,	38	
9	20-9-36	26-4-37	,,	218	Pair—reared specimens—fed with sugarcane stem and kept in dark chamber.
10	21-9-36	24-4-37	Female	215	
11	21-9-36	10-5-37	Male	231	Reared pair—fed with cane stem—and kept in dark chamber.
12	20-9-36	10-7-37	Female	293	
13	24-9-36	9-5-37	Male	227	Reared pair—fed with cane stem—and kept in dark chamber.
14	24-9-36	21-12-36	Female	88	
15	20-9-36	21-12-36	Male	92	Reared pair—male escaped—kept in wire-gauze cage in the open; cane fed.
16	16-10-36	25-1-37	Female	101	
17	26-10-36	13-12-36	Male	48	Reared pair—kept in wire gauze cage in the open with dung for egg-laying and Agave plant for feeding.
18	26-10-36	21-12-36	Female	56	
19	26-10-36	12-12-36	,,	47	Reared specimens—cane fed—male escaped—kept in glass jar.
20	26-10-36	1-3-37	Male	126	
21	26-10-36	31-5-37	Female	217	
22	28-10-36	19-3-37	,,	142	Reared specimens—cane fed—in glass jars.
23	28-10-36	25-3-37	Male	148	
24	28-10-36	15-5-37	Male	199	Reared specimens; cane fed in jars; male escaped.
25	28-10-36	31-5-37	Female	215	
26	28-10-36	11-6-37	Male	226	
27	28-10-36	17-4-37	Male	171	Reared specimens—cane fed in jars.
28	28-10-36	18-3-37	,,	141	
29	5-2-37	13-5-37	Female	97	
30	5-2-37	12-6-37	Female	127	
31	17-5-37	13-9-37	Male	119	Reared pair—cane fed—kept in glass jar.
32	17-5-37	1-9-37	Female	107	
33	17-5-37	12-9-37	Male	119	Ditto.
34	12-6-37	7-9-37	Female	87	

TABLE VIII—*contd.*

Serial No.	Date of capture	Date of death	Longevity in days	Remarks
35	21-12-36	16-5-37	Female 147	Collection from outside—date of emergence not known kept in dark chamber—cane fed.
36	27-9-36	9-5-37	Female 224	Specimen collected from tree—left without male in jar and cane fed—date of emergence not known.

STUDY OF SEASONAL INCIDENCE

In a plot with sixty-eight young trees the seasonal incidence of the pest has been under observation. The beetles in a dozen trees specifically marked were being examined and systematically hooked out every week, and at the same time in the same area two small manure pits 4 ft. × 4 ft. × 4 ft. were also dug out and kept in suitable condition for the beetles to come and lay eggs. The pits were examined alternately once a week to note the presence of the beetle or eggs in the dung. As the dung got dry fresh dung was added periodically so as to maintain always a suitable semi-wet condition for egg-laying by the beetles. The record of the beetles removed from trees and those along with eggs, if any, from pits, is given in Table IX.

TABLE IX

Incidence of the beetle in field trees and pits

Month	Number of beetles in dozen trees			Beetles, eggs and grubs in pits
	Female	Male	Total	
May 1936	1	..	1	1 female ; eggs *nil.*
June ,,	4	5	9	1 female ; eggs *nil.*
July ,,	5	3	8	*Nil.* *Nil.*
August ,,	3	1	4	2 females ; 1 male ; 24 eggs.
September ,,	3	1	4	1 female ; 74 eggs.
October ,,	6	1	7	*Nil.* *Nil.*
November ,,	7	3	10	1 female ; eggs *nil.*
December ,,	7	3	10	1 female ; 16 eggs.
January 1937	5	1	6	1 female ; eggs *nil.*
February ,,	14	5	19	*Nil.* *Nil.*
March ,,	20	15	35	*Nil.* *Nil.*

TABLE IX—*contd.*

Month	Number of beetles in dozen trees			Beetles, eggs and grubs in pits
	Female	Male	Total	
April 1937 .	31	15	46	1 female ; 44 eggs.
May „ .	13	9	22	*Nil.* *Nil.*
June „ .	2	2	4	*Nil.* *Nil.*
July „ .	5	3	8	3 females ; 1 male ; 173 eggs ; 3 grubs.
August „ .	8	3	11	2 females ; 2 males ; 69 eggs ; 14 grubs.
September „ .	1	2	3	5 females ; 3 males ; 68 eggs ; 10 grubs.
October „ .	5	2	7	
November „ .	14	6	20	
December „ .	14	7	21	2 females ; 1 male ; 163 eggs.
January 1938 .	14	4	18	4 females ; 2 males ; 96 eggs ; 16 grubs.
February „ .	10	8	18	1 female ; 1 male ; 15 eggs ; 10 grubs.
March „ .	12	13	25	3 females ; 2 males ; 135 eggs ; 63 grubs.

It will be seen from the observations during a period of nearly two years that the beetles are active throughout the year and the egg-laying is also known for all the months with few exceptions. Broods overlap without any definite sequence as the beetles are capable of living for a long time and laying eggs intermittently, and as grubs of different stages are noted almost throughout the year. While some of the beetles begin laying eggs earlier, a good number appear to prefer a longer period of active feeding before evincing breeding activity. It is also clear, that beetles visiting trees are more numerous during March and April. The same observations have been borne out by Ramchandra Rao [1930] who mentions that the attack is worst in the dry season from December to May.

NATURE AND EXTENT OF DAMAGE

Unlike many other forms where damage to crops is caused by the larval stages, in this instance, it is the adult insect that works havoc. The beetles after emergence soon find suitable shelter and food in the tender crowns of the coconut trees. Young as well as old trees are visited by these beetles. They actively burrow into living tissue in the topmost tender regions of the stem, getting access through the axils of the leaves, or cutting through the unopened leaves. The beetle holes, about 1 in. in diameter, vary from $\frac{1}{2}$ to 2 ft. in depth. Sometimes the leaf stalk itself is bored through longitudinally. Often the central shoot with one or two unfolded leaves is cut through drilled vertically down and, as a result, the leaves break off when they grow

FIG. 1. Three trees of the same age, one showing stunted growth due to repeated beetle attack

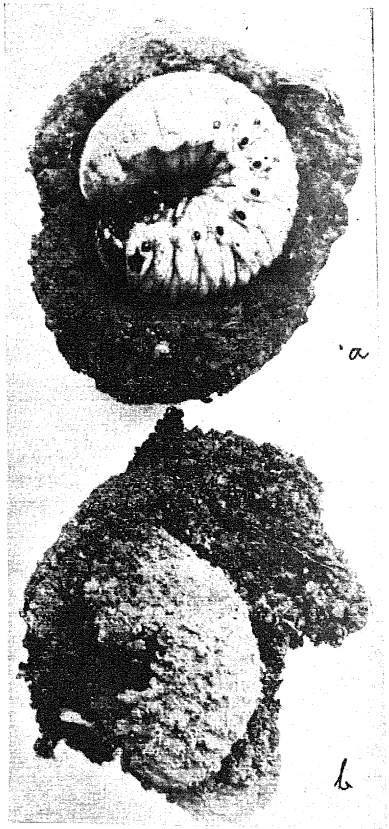

FIG. 2. (*a*) Healthy grub of *Oryctes rhinoceros* ; (*b*) Grub of *O. rhinoceros* affected by green muscardine fungus

up and open out, and bear marks of the beetle damage in a characteristic manner. In the case of the seedlings and small trees at times of beetle abundance, successive growths of central shoots are hampered to the extent of killing the trees or causing a definite setback in their growth, by repeated attacks (Plate XXIV, fig. 1). Mackie [1917] records that about one per cent of the trees are actually killed annually due to the beetle attack in the Philippine Islands. Death to the trees, caused by the unaided efforts of the beetle alone, is rather difficult of verification and computation, as the attacked trees take a long time before they are killed, and during the period other influences are at work. At the Insectary, two coconut seedlings about two years old had been kept under observation inside separate wire-gauze cages wherein beetles had also been liberated. In both cases, in the course of a year the seedlings were completely killed by the beetles. In nature, in view of the fact, that often four to six beetles are noted in a single tree at different axils and that beetles re-infest trees repeatedly in quick succession, substantial damage to foliage and growing stem is caused. In rare cases, the spathes are also attacked, resulting in reduction of nuts. Apart from such direct mechanical injury it is reported that as a result of the beetle damage, rain water collects in the excavations at the top, causing rotting. It is also indicated by Ghosh [1911], Hutson [1933] and others that the fresh wounds caused by the beetles attract to the tree the palm weevil which is capable of breeding in the tree itself. But, in South India, though beetle activity in varying degree of intensity is seen throughout, the weevil is less common. Our observations on the weevil also suggest that the reverse is always true, *i.e.*, a weevil-attacked tree is sooner or later a definite source of attraction to the beetle.

Food plants other than coconut in India

Fletcher [1914] has recorded the pest on palms of various species, American aloes and sugarcane. Ghosh [1923] gives a detailed list of palms infested by the beetle. In South India, the coconut palm is the most attacked, though the palmyra and the Talipot palms are also affected to some extent. Recently, the beetle has been noticed as doing damage to pineapple by E. R. Gopala Menon at Taliparamba. Agave is also another plant attacked by this beetle ; when plenty of palms are available in the neighbourhood this plant is rarely attacked.

Control methods tried

The very fact that various methods have been tried from time to time by various workers suggests that no one method has proved entirely satisfactory or suited for universal application. The following were tried at Coimbatore :—

1. Trial of baits.—Several kinds of baits namely, sweetened mash of oil-cakes—such as groundnut, castor, gingelly, coconut—with molasses, both in semi-solid and liquid conditions and allowed to ferment in earthen pots were tested. The beetles were not found trapped in any of these.

2. Insecticidal trials.—Granulated calcium cyanide was applied in small quantities to the bore holes containing beetles in young coconut trees. The beetles were found killed, but on such trees it produced slight damage by way of scorching or discolouration to the leaves, especially the tender shoots. As such the material is found unsuitable.

3. Use of light traps.—During periods of abundance of the beetles they dash against street lights but they do not seem to be attracted to specific light traps in any number to be of economic value as control.

4. Trap pits.—Removal of beetles and their immature stages from breeding material, such as manure heaps, dead coconut stumps, etc., has been suggested by many. This method is no doubt of some economic value but has its own drawbacks. In a small area, with sixty-eight young trees, there were two trap pits containing dung. The beetles, eggs and larvae, were removed from each of these pits, at intervals of a fortnight systematically. From May 1936 to March 1938, as many as 42 beetles, 877 eggs and 116 grubs were removed, but there was no material reduction in the damage to the trees, due to the influx of beetles from breeding places elsewhere. The destruction of beetle stages in breeding material in trap pits is not likely to confer much protection to individual gardens unless control measures are taken over a large area.

5. Use of beetle rods.—An iron rod, about 2½ ft. long, 3/8 in. thick and with a hook at one end and the other end bent so as to form a ring for gripping serves fairly satisfactorily for easily locating and removing the beetles from holes. In rare cases, the bore holes run across stem or petiole, when the beetle remaining there is not easily dislodged. In such cases, the side fibrous mattings covering the base of petioles have to be disentangled and the leaf base exposed, to reach the beetle which is then hooked out. As a protection against further entry of beetles into the holes, the holes are filled with sand.

Natural enemies

A mite (*Uropoda* sp.) is sometimes found in large numbers infesting the beetle but no beetle was found actually killed by the mite attack. In rare cases, the mites attack the pupal stage and are able to weaken the emerging adult.

Ants in manure pits attack pupae in imperfectly formed cells and bring about casualties to the same. But the greater mortality to larval and pupal forms, appear to be due to causes, such as overcrowding, want of suitable situation for pupation and drying up of the dung.

Crows are fond of preying on the beetle grubs. When the contents of a manure pit are spread out, crows collect in the area in numbers and remove all exposed grubs.

The green muscardine fungus.—Smith [1936] from Ceylon has described the method of controlling the larvae in breeding places by utilising the green muscardine fungus. Hopkins [1927] has reviewed the work of Friederichs in the introduction of natural enemies in Samoa including the green muscardine fungus for the control of the beetle. Though the early introduction

of the fungus in Ceylon was not reported to be a success, it is now recognised in Ceylon as one of the effective means of checking the pest. Through the courtesy of the Department of Agriculture in Ceylon, the fungus culture was got for trials in South India during 1937. It is found that the fungus is easily raised in culture to desired quantities under laboratory conditions. The trials on the activity of the fungus in pots as well as in pits have indicated that (*i*) the fungus is able to fatally attack the larvae and pupae, (*ii*) the eggs are practically unaffected and are able to hatch out in the inoculated material, (*iii*) the adult beetles continue to thrive and lay eggs in the same, (*iv*) the young grubs are also affected but are difficult to be detected when attacked and (*v*) the full-grown grubs show signs of discolouration at the initial stage of attack and later become covered with the growth of the fungus (Plate XXIV, fig. 2). In view, however, of the great powers of flight of the beetles and the variety of situations under which they breed in different localities, the success of the method is limited to the extent to which they breed in dung and the difficulty cited elsewhere in the case of ordinary dung trap pits has to be overcome here too. The viability of the fungus under periods of drought, the maximum period of the longevity of the spores or resting stage, if any, under adverse conditions, and the possibility of the fungus being multiplied in dung, are being studied.

Acknowledgments

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APPEN

Temperature and humidity records during the

	December 1935	January 1936	February 1936	March 1936	April 1936	May 1936	June 1936	July 1936	August 1936	September 1936	October 1936
Mean maximum temperature in shade (° F.).	84·9	87·8	91·0	94·1	95·5	98·7	85·4	86·1	85·8	89·9	87·7
Mean minimum temperature in shade (° F.).	65·8	64·4	67·9	70·5	72·8	74·8	71·6	71·1	71·2	71·2	70·5
Mean humidity at 8 hours (per cent)	75·1	73·8	75·4	73·4	69·2	71·4	70·4	72·1	73·9	75·5	79·2

DIX

period of study of the coconut palm beetle

November 1936	December 1936	January 1937	February 1937	March 1937	April 1937	May 1937	June 1937	July 1937	August 1937	September 1937	October 1937	November 1937	December 1937
83·9	84·6	86·0	91·2	94·1	93·5	93·7	89·2	85·3	88·7	89·5	86·9	82·2	85·0
69·0	66·8	64·0	68·2	70·8	73·0	73·7	75·8	74·6	71·2	70·8	70·6	69·0	68·9
84·0	76·0	72·8	75·5	70·2	71·5	72·7	68·5	72·0	74·5	72·0	77·4	81·4	74·3

CHROMOSOME NUMBER IN INDIAN LINSEED, *LINUM USITATISSIMUM* L.

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(Received for publication on 6th September 1938)

(With Plate XXV)

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I. Introduction

The Indian linseed, *Linum usitatissimum* L., has been investigated especially from economic points of view by several workers in India [Howard, Howard and Khan 1910, 1920; Graham and Roy, 1924; Howard and Khan, 1924; Shaw, Khan and Alam, 1931; Deshpande and Mallik, 1937; Kadam, Kulkarni and Patel, 1938]. Cytologically the varieties have not so far been studied in this country. The present paper deals with the somatic and meiotic chromosomes of some varieties grown in the Central Provinces and Berar.

The diploid chromosome number in the varieties of *Linum usitatissimum* examined in other countries by Reynders [1926]*, Kikuchi [1926]*, A. Nikolajewa [Emme and Schepeljeva, 1927]* was found to be thirty and haploid number by Vilmorin and Simonet [1927]* and Kikuchi to be fifteen. But Emme and Schepeljeva record thirty-two as the diploid number in a great number of different varieties of this species and thirty in only one variety. Martzenitzina [1927]* also records thirty-two as the diploid number in this species.

* These references have been taken from " Tammes [1928], who has reviewed the literature on the genetics of the genus *Linum*.

II. Material and technique

For the investigation of the somatic chromosomes the seeds were selected from four improved strains and two local samples which exhibited variations in colour and size of seeds as shown in Table I.

Table I

Linseed varieties used in the studies of the somatic chromosomes

Serial No.	Variety	Colour	Size
1	Local-Chanda (Baroda)	Fawn (Plate XXV, fig. 2).	Bold
2	Local-Balaghat (Waraseoni) . . .	Brown . . .	Medium
3	E. B. 3 (Selection)	Brown . . .	Bold
4	No. 117 (Selection)	Yellow (Plate XXV, fig. 3).	Bold
5	No. 3243 (E B 3× Punjab Linseed) .	Brown (Plate XXV, fig. 1).	Bold
6	No. 3255 (E B 3 × Punjab Linseed) .	Brown . . .	Bold

The root-tips which were ready for fixation invariably on the third day were fixed in S. Navashin's chromo-acetic-formalin fixative [Navashin. M, 1934] made in the proportion shown below, and followed by the usual paraffin embedding. The sections were cut at 12-15 μ followed by the iodine-gentian-violet staining.

1 per cent chromic acid	10 c.c.
20 per cent formalin	4 c.c.
(Original formula contains 16 per cent formalin)	
Glacial acetic acid	1 c.c.

The fixation and the staining were satisfactory. After a period of nearly six to seven months the preparations showed some destaining.

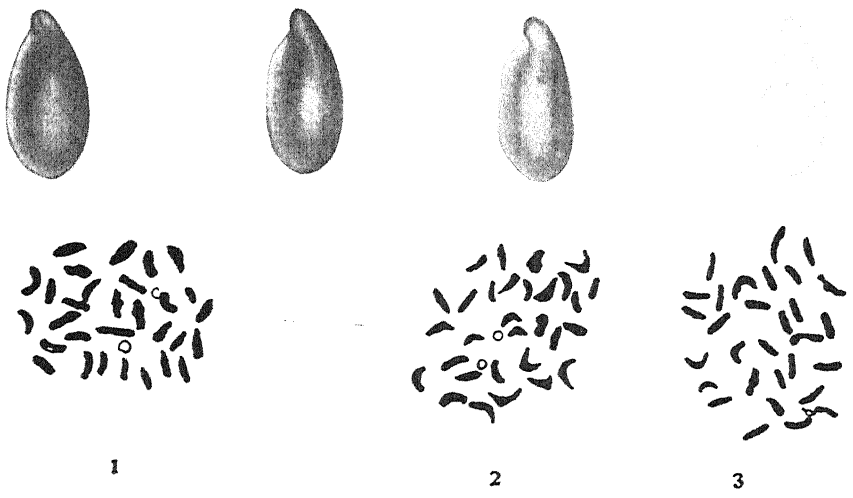
For the meiotic chromosomes the smears of pollen mother cells were obtained from plants raised out of the local material. They were fixed in La Cour 2 B E followed by the iodine-gentian-violet staining.

All figures were drawn at the magnification of × 3,200.

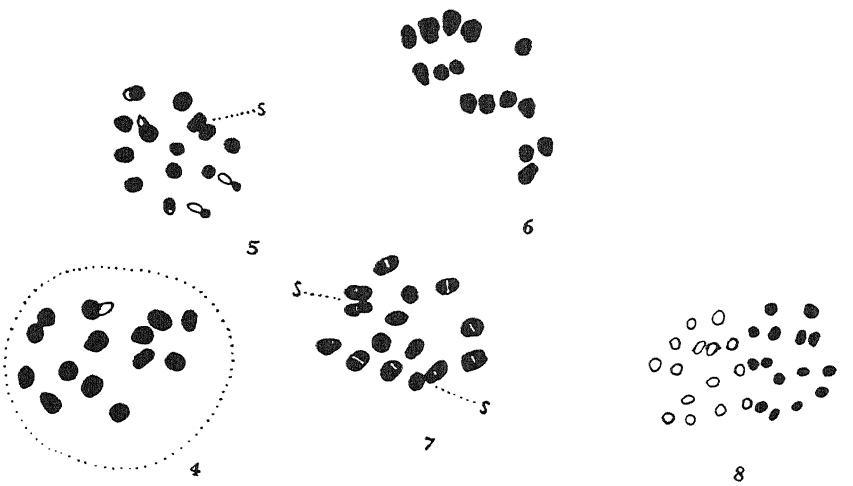
III. Observations on somatic chromosomes

1. Local-Chanda (Baroda)

Twelve well-spread plates were examined which showed thirty chromosomes. Occasionally one or two nucleolar fragments were observed which were found lying either in the midst of the chromosomes or away in the surrounding cytoplasm. Some pairs of chromosomes exhibit a sort of terminal affinity.



1. No. 3243 (E B 3×Punjab linseed) with two nucleolar fragments observed at different levels (2 n=30); 2. Local-Chanda (Baroda) with two nucleolar fragments observed at different levels (2 n=30); 3. No. 117 (Selection) with one nucleolar fragment seen at a different level (2 n=30)



4. Late diakinesis showing darkly stained bivalents, seen scattered at different levels ; 5. I metaphase, polar view, showing 15 bivalents ; 6. A stage in-between pro-metaphase and metaphase ; 7. I metaphase showing 15 bivalents ; 8. II metaphase plates in different planes with slanting positions, shown in black and empty circles

(S=Secondary association)

The chromosomes on the whole are very short and to some extent they show among themselves some morphological variations, such as the size (large and short) and shape (rod-like and bent). The various shapes are probably due to the different positions of the attachment constrictions (Plate XXV, fig. 2).

2. No. 3243 (E B 3 × Punjab linseed)

Thirty chromosomes are distinctly observed. The plates invariably showed two nucleolar fragments. The circular shape of the fragments and their positions at slightly different levels from the mitotic plate help in isolating them from the small types of chromosomes. The plate (Plate XXV, fig. 1) shows distinctly most of the homologous chromosomes lying more or less in parallel positions.

3. No. 117 (Selection)

$2n=30$ chromosomes were distinctly observed. Only one nucleolar fragment at different focus was seen (Plate XXV, fig. 3), which was always found lying very close to one of the chromosomes.

4. Local-Balaghat (Waraseoni Red)

It showed $2n=30$ with occasional one or two nucleolar fragments which sometimes are found associated with chromosomes.

5. E B 3 (Selection)

It showed $2n=30$ with one nucleolar fragment. The latter was always seen at a different level.

6. No. 3255 (E. B. 3 × Punjab linseed)

$2n=30$, with one or two nucleolar fragments, are distinctly observed, The latter are the circular bodies situated at different levels.

Morphologically the chromosomes in each set can be distinguished into three distinct types.

1. Long and rod-like chromosomes.
2. Short and rod-like chromosomes.
3. Bent chromosomes.

Further morphological distinction is difficult.

IV. Observations on meiotic chromosomes

Meiotic chromosomes were examined from the permanent smear preparations at late diakinesis, I metaphase and II metaphase.

At late diakinesis fifteen bivalents were seen all scattered at different levels (Plate XXV, fig. 4). On account of deep staining it was not possible to see distinctly the chiasmata in the bivalents. No nucleolar material is detected, The nuclear wall completely disappears at this stage.

At I metaphase fifteen bivalents are distinctly seen in polar view (Plate XXV, figs. 5 and 7). Nucleolar material is not observed. Secondary associations are formed at this stage (Plate XXV, figs. 5 and 7, S) suggesting thereby that linseed is a secondarily balanced polyploid. Plate XXV, fig. 6 probably represents a stage in between the pro-metaphase and metaphase.

At II metaphase fifteen univalents are observed (Plate XXV, fig. 8).

V. Summary

The somatic and meiotic chromosomes of Indian linseed, *Linum usitatissimum* L., grown in the Central Provinces and Berar were examined. The diploid chromosome number is found to be thirty in root-tip cells.

At late diakinesis and I metaphase fifteen bivalents and at II metaphase fifteen univalents are observed.

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COMPARISON OF DIFFERENT METHODS OF COMPOSTING WASTE MATERIALS*

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WORK has been proceeding at the Indian Institute of Science for some time past on a comparison of different methods of composting town and farm wastes. Special attention was paid to the ' hot ' fermentation method, wherein compost material was kept aerobic for a preliminary period of five to six days, during which time, there was rapid decomposition and rise of temperature to 65-70°C. At the end of the above period, the mass was covered over with a layer of mud paste and over it earth and kept closed for about $2\frac{1}{2}$ to 3 months, to allow anaerobic rotting to take place. The manure was then ready for application to land. The hot fermentation method was compared with the aerobic methods in ordinary use in this country, *e.g.*, decomposition in shallow wide trenches and in heaps overground.

The following procedures of composting were compared using one ton lots of compost material.

1. Hot fermentation in underground trenches which were brick-lined and plastered.

2. Hot fermentation in underground trenches without brick-lining.

3. Hot fermentation method in heaps overground on brick-lined platforms.

4. Hot fermentation method in heaps overground on platforms which were not brick-lined.

5. Aerobic process in shallow wide trenches which were not brick-lined.

6. Aerobic process in heaps overground on platforms which were not brick-lined.

The quantities of materials taken and the methods of mixing were the same for the hot fermentation and aerobic methods. The hot fermented composts, however, were packed air-free after five or six days as described above, whereas the ' aerobic ' composts were turned over once a fortnight and watered if necessary.

Two different starters were tried, *viz.*, night soil and urine-dung mixture. Each trial was carried out in duplicate.

*Contribution to the Discussion on " Composts " held by the Indian Science Congress, at Lahore in January 1939.

In the first series using night soil as starter, the relative proportions of the constituents taken were 1000 lb. of screened leaves and other organic fractions of street sweepings, 500 lb. of the screened soil fraction of street sweepings and 1000 lb. of night-soil (fresh). These were mixed in aliquot portions at a time within the container itself, where the compost was to be kept. The moisture level was brought to about 50 per cent by the addition of seventy gallons of water, which were added to the night-soil in portions, so as to facilitate uniform mixing of the street sweepings with night-soil. The mixing operations were generally carried out with forks.

In the second series, where urine-dung mixture was used as the starter, an emulsion of dung and urine was prepared by the addition of 200 lb. of dung to twenty-five gallons of cattle urine, and out of this emulsion, five gallons (by volume) were uniformly mixed with every lot of 100 lb. of leaves and 50 lb. of soil fraction. Further water to the extent of four to five gallons were added. The mixing was done within the container itself in small lots of the above order, which were continued till a total weight of 1000 lb. of leaves and 500 lb. of soil had been taken.

All the manures were removed after three months, and weighed. Samples were taken for analysis and the main bulk was stored in covered cisterns till required for pot and plot experiments.

The refuse materials used for composting had the following average composition :—

Composition of refuse materials

(*All figures are on fresh basis including moisture*)

	Street-sweepings screened into		Night soil (fresh)
	Soil fraction (per cent)	Leaves fraction (per cent)	(per cent)
1. Total carbon	3·07	30·71	9·30
2. Total nitrogen	0·24	1·23	1·10
3. Ash-free organic matter	6·20	54·30	15·30
4. Ash	91·30	36·10	4·20
5. Moisture	2·50	9·60	80·50

The analytical data obtained are presented in Tables I and II, the former referring to the experiments with night soil and the latter to those where urine plus dung was used as the starter.

TABLE I

Semi-large scale experiments using night soil as starter

Taken 1000 lb. leaf material 500 lb. soil fraction of town refuse 1000 lb. night soil (fresh)	Yield of manure		Carbon		Nitrogen		Ash-free organic matter	
	Fresh lb.	Sun-dried lb.	Per cent analysis	Per cent recovery	Per cent analysis	Per cent recovery	Per cent analysis	Per cent recovery
I. Hot fermentation in underground Trenches with brick-lining.	3,215	1,520	19·81	72·46	1·22	75·91	34·93	73·05
II. Hot fermentation in underground trenches without brick-lining .	3,010	1,438	18·01	62·34	1·13	66·36	31·85	63·01
III. Hot fermentation in heaps overground on brick-lined platforms.	2,760	1,325	17·84	56·90	1·19	64·34	30·39	55·39
IV. Hot fermentation in heaps overground on platforms not brick-lined.	2,620	1,280	17·05	52·53	1·10	57·58	29·55	52·02
V. Aerobic, in shallow wide trenches	2,230	1,185	16·60	47·36	1·08	52·49	29·36	47·87
VI. Aerobic in heaps overground .	1,980	1,042	14·98	37·58	0·99	42·09	28·89	41·41

TABLE II

Semi-large scale experiments using cattle urine and dung as starters

Taken 1,000 lb. leaf material, 500 lb. soil, 50 gallons of urine-dung mixture	Yield of manure		Carbon		Nitrogen		Ash-free organic matter	
	Fresh	Sun-dried	Per cent analysis	Per cent recovery	Per cent analysis	Per cent recovery	Per cent analysis	Per cent recovery
	lb.	lb.						
I. Hot fermentation in underground trenches with brick-lining.	3,015	1,415	17·81	77·45	0·77	79·52	31·11	75·88
II. Hot fermentation in underground trenches without brick-lining.	2,784	1,320	17·04	69·15	0·74	71·53	28·78	65·52
III. Hot fermentation in heaps overground on brick-lined paltforms.	2,645	1,275	16·47	64·54	0·69	64·12	26·27	57·76
IV. Hot fermentation in heaps overground on platforms not brick-lined.	2,496	1,190	15·13	55·32	0·63	54·75	25·66	52·64
V. Aerobic, in shallow wide trenches	2,112	1,075	13·95	46·10	0·60	47·10	24·82	46·03
VI. Aerobic, in heaps overground .	1,826	960	13·02	38·41	0·59	41·35	23·44	38·80

The data clearly confirm the superiority of the hot fermentation process over the aerobic methods, as shown by the higher conservation of carbon, nitrogen and of ash-free organic matter.

Considering first the experiments with night soil, recoveries of carbon, nitrogen and ash-free organic matter as high as 72-76 per cent can be obtained by adopting the hot fermentation principle in trenches which are brick-lined and plastered. The recovery, however, falls to about 62-66 per cent when the trenches are not brick-lined and plastered, due probably to seepage losses. In heaps overground on brick-lined platforms which are closed by a layer of earth after the first week (*vide* treatment III) the recovery obtained is of the

order 57-64 per cent and in the heaps overground on platforms which are not brick-lined but which are similarly closed after the first six days, according to the hot fermentation principle (*vide* treatment IV), the recovery is about 52-57 per cent. The recoveries are lowest in the aerobic methods, whether conducted in shallow trenches or in heaps overground. The conservation is rather better in the former case, being about 47-52 per cent, as compared with about 40 per cent recovery in the latter case.

A similar picture is shown by the data relating to experiments where urine plus dung was used as the starter. The recoveries of carbon, nitrogen and of ash-free organic matter are particularly good in the case of the hot fermentation methods, being of the order of 65 to 80 per cent, while the aerobic methods show a recovery of only 38-47 per cent.

The low recoveries in the aerobic processes are, no doubt, due to the more vigorous oxidation and decomposition of the constituents due to turning the mass over at intervals, accompanied by watering. It is no doubt true that for the preparation of satisfactory compost there should be an appreciable amount of oxidation and biological decomposition of the organic refuse. Such preliminary decomposition serves to remove the more easily oxidisable constituents which, otherwise, may later retard nitrification when applied to the soil. But it is quite possible that the degree of preliminary oxidation may be accentuated in some of the aerobic methods now in use and the biological decomposition prolonged to such an extent that even the less easily oxidisable constituents such as the cellulose-xylan complexes and ligno-cellulose may be attacked. These complexes, on account of their slow rate of decomposition, will not interfere with nitrification in the soil, but may, on the other hand, help to increase the amount of soil organic matter. Certainly, a stoppage of the aerobic type of decomposition, at an earlier stage, means a proportionately greater recovery of manure.

But in addition to stopping the aerobic decomposition at an earlier stage, the hot fermentation method requires the maturing of the manure under anaerobic conditions for $2\frac{1}{2}$ to 3 months, during which period the material undergoes further degradation and humification, and its nitrogenous compounds are rendered more easily available for nitrification.

In this connection, it may be mentioned that there is a strong school of opinion who doubt the wisdom of composting refuse materials with nitrogenous starters and enquire whether a similar benefit may not be obtained by an application of the refuse material directly on to land, along with the amount of nitrogenous starter required to successfully compost it. Experiments on these lines have been recently started at Rothamsted [1935, 1936, 1937], but the results obtained, so far, are of a preliminary and conflicting nature. Eden [1937] has also started in Ceylon a series of experiments to compare the effect of the application of prunings and other tea garden wastes directly on to the land carrying the crop, instead of previously composting the same before application. These experiments also have only recently been begun and have not yielded so far any definite results.

The above school of thought lay emphasis on a suitable C : N ratio in the refuse material added to the land, rather than on the chemical make-up of such material. One of the striking features of composting is the alteration in chemical make-up of the organic wastes, by way of removal of the readily oxidisable constituents, in addition to the shifting of the C : N ratio into a more optimum range for crop production. The results of the above experiments carried out at Rothamsted and in Ceylon, if continued for a decade or more, will no doubt, help in differentiating between the beneficial effects due to such alteration in chemical make-up brought about by composting and those due to the presence of an optimum C : N ratio. Interesting results may be obtained if experiments on similar lines could be conducted in this country, since the climatic and soil conditions here vary greatly from those in England, and the Ceylon experiments are confined only to tea estate wastes, which have a narrow C : N ratio (about 12 : 1).

The hot fermentation method strikes a *via media* between the above school of thought and the followers of the older school of aerobic composting, in that the readily oxidisable constituents are removed, in the hot fermentation system, during the preliminary period of aerobic decomposition and, further, the availability of nitrogen is increased by the higher percentage of nitrogen secured in the final compost. Plot experiments in the field and nitrification studies in the laboratory, to be described below, have shown that the hot fermented manure is equal in quality and, in many trials, superior to the aerobically prepared manure.

Nitrification studies in the laboratory

As typical representatives of the three methods of composting, the hot fermented compost in trenches, the aerobically prepared manure in shallow trenches and the material which was anaerobically packed from the start, were taken for nitrification studies in the laboratory. Two grm. portions of the fresh manure were added to 100 grm. portions of a garden soil from Bangalore, in wide-necked bottles, mixed well and enough water was added to bring the moisture content to 50 per cent of the water-holding capacity. The bottles were weighed and every alternate day the requisite amount of water was added to bring back the moisture content to the initial level.

At the end of 4, 6, 8, 12 and 16 weeks, duplicate bottles for each treatment were removed, the contents were air-dried, weighed, and aliquots were taken for the determination of (*a*) ammoniacal nitrogen (*b*) total nitrogen and (*c*) nitrate nitrogen. As the figures for total nitrogen and ammoniacal nitrogen

did not show any striking variations between the different treatments, only the data for nitrate nitrogen are presented in Table III.

TABLE III

Nitrification experiments in the Laboratory

Nature of manure (added 2 grms. fresh manure to 100 grms. graden soil)	Mg. of NO_3-N present per 100 grms. of soil after incubation for				
	4 weeks	6 weeks	8 weeks	12 weeks	16 weeks
1. Hot fermentation in underground trenches	4·52	5·05	5·59	6·38	6·92
2. Aerobic process in shallow wide trenches	3·72	3·99	4·52	5·05	5·32
3. Anaerobic packing from the start .	3·46	3·72	4·52	5·32	5·85

The figures show that the hot fermented compost nitrifies at a more rapid rate than the aerobically prepared manure ; this is partly due to the high content of nitrogen in the former manure. It is interesting to note that the initial rate of nitrification of the material preserved anaerobically from the start is lower than that of the other two. This is probably due to the fact that such anaerobically kept material contains a good amount of the easily oxidisable fraction which retards nitrification in the soil. In the hot fermentation method, such easily oxidisable fractions are removed during the preliminary aerobic treatment.

Plot experiments

Plot experiments were carried out to determine the manurial value of the night soil composts prepared by the different methods. The amounts of the different manures corresponding to oven-dry weights of 20 lbs. were added to plots 10 ft. × 10 ft. in random arrangement. The crops tried were *ragi* (*Eleucine coracana*) in the first season and *jowar* (*Sorghum vulgare*) for residual effect in the succeeding season. The soil used was a sandy loam, rather poor in nitrogen and phosphorus.

The crop yields obtained are given in Table IV. They again demonstrate the superior manurial value of the hot fermented composts over others. This superiority is no doubt due to the better conservation of nitrogen secured in the hot fermentation method.

TABLE IV

Results of plot experiments with composts—7 × 7 Latin Square
(ragi *in the first season and* jowar *in the next season for residual effect*)

Treatment (composts prepared from night soil and town refuse)	*Ragi* (*Eleucine coracna*)				*Jowar* (*Sorghum vulgare*) fodder (dry wt.)	
	Grain		Straw		Totals of 7 plots	Yield per acre
	Totals of 7 plots	Yield per acre	Totals of 7 plots	Yield per acre		
	lb.	lb.	lb.	lb.	lb.	Tons
I. Hot fermentation in underground trenches bricklined and plastered . .	43·0	2,676	93·8	5,837	441	12·26
II. Hot fermentation in underground trenches without bricklining . .	38·8	2,414	88·2	5,489	392	10·89
III. Hot fermentation in overground heaps plastered over with mud . .	36·7	2,284	86·1	5,358	354	9·84
IV. Aerobic method in shallow wide trenches . .	32·2	2,004	80·5	5,009	322	8·95
V. Aerobic method in heaps overground . . .	30·1	1,874	75·6	4,704	294	8·17
VI. Ammonium sulphate of equivalent nitrogen content	27·3	1,699	71·7	4,462	210	5·83
VII. Control plots—no manure	18·2	1,133	44·1	2,745	161	4·47
Mean yield of treatments .	32·3	2,012	77·1	4,801	311	8·64
Standard deviation .	1·49	92·7	2·33	145	16·27	0·45

The work described above was carried out as part of a scheme of investigation on composting methods carried out at this Institute with the financial assistance of the Imperial Council of Agricultural Research. The writer wishes to acknowledge help received from Mr. C. Parthasarathy in carrying out the nitrification studies in the laboratory.

SUMMARY

A comparison of different methods of composting town and farm wastes, as judged by plot trials in the field and by nitrification experiments in the laboratory, showed the superiority of the 'hot' fermentation process over the usual aerobic methods. The former gave a higher recovery of organic matter and of nitrogen than the latter (about $1\frac{1}{2}$ times) and the resulting manure was equally efficient in crop production and in several cases superior. Starting from the same amount of original materials, it is possible, by suitable dilution with soil, to obtain by the hot fermentation process about $1\frac{1}{2}$ times as much manure as is obtained by the usual aerobic methods and containing the same percentages of carbon and of nitrogen. Indications were obtained during the course of the work that the rationale of composting waste materials needs a re-examination. The satisfactory behaviour of the hot fermented composts in the field would go to show that in the aerobic methods, as usually adopted, the oxidation is carried to an extreme stage when even the less oxidisable fractions are attacked and thus avoidable losses of dry matter and of nitrogen occur. The recent experiments at Rothamsted and in Ceylon on composts *versus* uncomposted material are also discussed.

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REVIEWS

Science in Africa. By E. B. Worthington : Oxford University Press, London, New York, Toronto ; 1938. Pp. XVI & 746. Price 10*s*. 6*d*.

This book is one of the issues of the African Research Survey conducted under the guidance of Lord Hailey with the object of exploring further possibilities for the development of the continent. The author had the privilege of joining Lord Hailey during the latter part of the tour and thus acquainting himself with the conditions prevailing there in addition to his previous experience gained in East Africa from his work in connection with the Great Lakes.

In this book, Dr. Worthington deals in a thorough manner with the present organisation and results achieved in different branches of science and gives the reader a comprehensive picture of the position attained and progress made in the various subjects.

It is difficult to find oneself in full agreement with all that is written ; page 421 being an example of the repetition of a superstition which was easily exploded in India though tenaciously held by the earlier workers. It is also difficult to visualise the potentialities of the beef breed aimed at—if the Hereford, Angus and Dexter-Kerry are all regarded—as equally suitable crosses. Plate VII is remarkable both for the straightness of the work and the curious method of training adopted. A double mould board being to say the least a curious implement for training purposes, but taking it by and large a variety of subjects are dealt with in considerable detail and the book forms an interesting addition to our knowledge of Africa. [W. S.]

International Bibliography of Agricultural Economics. (Quarterly publication of the International Institute of Agriculture, Rome).

The International Institute of Agriculture in Rome has now begun to publish a current bibliography, at quarterly intervals, which deals with agricultural economics in all its various phases. The first issue of October 1938 has just been printed.

The International Bibliography of Agricultural Economics covers the economic and social aspects of agriculture, such as agricultural economics, agricultural policy, settlement, credit, cooperation, insurance, marketing, prices, statistics, farm organisation and management, valuation, labour, accounting, rural sociology, agricultural history and geography, legislation and education and all other agricultural problems, in so far as they are considered from the economic and social point of view. Only publications of purely technical character are excluded. Titles of all publications, whether books, bulletins, pamphlets or articles in periodicals, are indicated, including all bibliographical details required for proper identification. All languages receive equal treatment and titles in the less known languages are provided with a translation.

The bibliography, which is the only one which covers systematically the world literature on agricultural economics, is carefully classified by subjects. An author-index will be supplied at the end of each volume. The annual subscription, postage included, is 6*s*. 6*d*. or $1,60.

ORIGINAL ARTICLES

STUDIES IN INDIAN PULSES

IX. CONTRIBUTIONS TO THE GENETICS OF *MUNG* (*PHASEOLUS RADIATUS* LINN., SYN. *PH. AUREUS* ROXB.)

The inheritance of colour in the flower, unripe pod and in the seed-coat in *mung* and the linkage relations of these characters to other qualitative and quantitative characters

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(With Plate XXVI and one text-figure)

I. INTRODUCTION

THE mode of inheritance of the colour of the flower and of the seed-coat has been studied by several investigators in a number of species of *Phaseolus*, *viz.*, in the common garden bean (*Ph. vulgaris*), in the Adzuki bean (*Ph. chrysanthos*), and the runner bean (*Ph. multiflorus*). The literature on the genetics of *Phaseolus* has been throughly reviewed by Kooiman [1931] and by Matsuura [1929] and hence no attempt has been made in this paper to recapitulate the same. Genetical studies in the green gram or *mung* (*Ph. radiatus* Linn.) do not appear to have been reported up to now.

The purpose of this paper is to present the results of an investigation, conducted in the Botanical Section of the Imperial Agricultural Research Institute, Pusa, on the inheritance of colour in the flower, unripe pod and seed-coat and the relationship of these characters among themselves and with certain quantitative characters, in a cross between a green-seeded and a yellow-seeded type of *mung*. It may be pointed out that the yellow-seeded types of *mung* usually fetch a higher price in the market but are mostly indifferent yielders, whereas the green-seeded varieties are usually very prolific. As a result of the present hybridisation work a number of high-yielding, yellow-seeded hybrids have been secured.

II. MATERIAL AND METHOD

Green gram or *mung* is one of the most nutritious of pulses, and in common with the other members of the *Leguminosae*, it forms an important crop in the rural economy of India. Forty different types of this crop, comprising four distinct varieties, have been isolated and described by Bose [1932] and a study of the root-system of all these types and of twenty-five types of the allied species, black gram, *Phaseolus mungo*, var. *Roxburghii* has been published by Bose and Joglekar [1933].

A cross was made in 1932 between Pusa Type 18 (dark-green seeded *mung* belonging to the variety *typica*) and Pusa Type 36 (a pale lemon-yellow seeded type falling under the variety *aureus*). The reciprocal cross was also attempted at the same time but no seeds were obtained.

Though hundreds of flowers were crossed, only one fertile pod was obtained. The *mung* flower is very delicate and sheds readily ; this probably is the reason for the poor setting obtained. Of course the hot dry weather prevailing at the time when the crop is in full bloom at Pusa, *viz.*, in March and April, may be another factor which contributes also to the failure in securing good setting.

The pollen is usually shed before the opening of the flowers and for the purposes of hybridisation emasculation should be done as soon as the buds are nearly full grown, but before the standards begin to open. The best rule appears to be to emasculate the buds one or two days before the anthers are mature and then to pollinate the flowers a day or two after this operation. Fertilisation is usually complete in a couple of days, when the growing pod may be seen emerging out of the floral envelope.

For purposes of comparison pure seeds of the parental types were always grown along with the different filial generations. In this experiment only one particular field (Northern Trial Ground No. 11) was used for three consecutive years. No manures were applied to the *mung* itself but this crop invariably followed paddy which had received a dressing of niciphos at the time of its transplantation. *Mung* was sown in or about the second week of February and was usually harvested by the following May or June.

F_1 Generation

Only two F_1 plants were obtained and were grown in the field in 1933. In Table I are presented some of the important characters of the F_1 plants as well as of the two parents.

Table I

Some differentiating characters in Pusa mung *Types 18 and 36 and in the F_1 hybrid plants observed in 1933*

Characters	Type 18	F_1 hybrids	Type 36
1. Habit . .	Semi-erect .	Semi-erect but with a tendency towards erectness.	Semi-erect
2. Maturity as measured by the average number of days taken to flower	Early (74 days) .	Very early (56 days)	Early (65 days)

TABLE I—*contd.*

Characters	Type 18	F_1 hybrids	Type 36
3. Stem colour .	Purplish green	Green with purplish splashes	Green with purplish splashes
4. Leaves . .	Green, medium in size	Green, medium in size	Light green, medium in size
5. Flower colour .	Light yellowish olive	Intermediate light yellowish olive	Olive yellow
6. Pod colour—unripe	Dark green with deep red veins on the sutures.	Green with red veins on the sutures	Green with no red veins on the sutures
7. Pod colour—ripe	Olive grey .	Olive grey	Olive grey
8. Average length of pod in cm.	6·33 ± 0·05	7·05 ± 0·13	7·30 ± 0·05
9. Average diameter of pods in mm.	3·42 ± 0·04	3·61 ± 0·03	3·81 ± 0·03
10. Seed colour and nature of seed-surface	Dark green, dull	Dark green, dull .	Pale lemon-yellow, shining

No clear-cut differences could be seen in the F_2 and F_3 plants in regard to habit, stem-colour and the size of the leaves. The foliage colour also was difficult to distinguish, the development of colour depending to a large extent on the environment and especially on the presence of patches of differential fertility in the field. These characters were not studied, therefore, in any detail. The colour of the ripe pods remained pure for olive grey and showed no segregation. The genic analysis was, therefore, confined to the colour of the flowers, the colour of the unripe pods and that of the seed-coat, and the nature of the seed surface, *i.e.*, whether the seed-coat was shining or dull. Three quantitative characters were studied, namely, maturity (as measured by the number of days taken by the first flower to make its appearance in each plant), the length and the diameter of the pods. The relationship of the colour characters to the quantitative characters just mentioned was also determined. It will be observed from Table I that the colour of the flowers, pods and seeds in Type 36 parent was lighter in tint than that in Type 18 parent.

F_2 AND F_3 GENERATIONS

Selfed seeds of the two F_1 plants were dibbled by hand, under field conditions, in rows one-and-a-half feet apart; the plants were spaced nine inches apart in the row. Thus two F_2 progenies were raised in 1934 from the seeds of each of these F_1 plants and these were denominated for convenience as series A and series B respectively. Single plant progenies of sixty-four selected F_2 plants from series B were grown in the F_3 generation in 1935.

III. Inheritance of qualitative characters

(*1*) *Colour of the flower and of the unripe pod*

The study of the flower colour in the present investigation has been restricted to the colour of the standard. Slight variations were observed also in the colour of the wings as well as in the keel, but as the differences were not always clear-cut, a detailed study of these characters was not attempted.

The parental types of *mung*, *i.e.*, Type 18 and Type 36, have light yellow olive and olive yellow flowers respectively, there being more of olive present in the flowers of Type 18 and more of a yellow tinge in Type 36. For naming colours, Ridgway's " Colour Standards and Colour Nomenclature " was used. The flowers in F_1 plants and plants heterozygous for flower colour in the following generations were characterized by the possession of a lighter tint of olive than that found in Type 18. Considerable care had to be taken to differentiate between the flowers of this intermediate phenotype and those of the Type 18 parent.

The colour of the unripe pods appears to be controlled by the same genes which are responsible for the inheritance of the flower colour and hence the behaviour of these two characters in F_2 and F_3 generations has been considered together. As already mentioned, the unripe pods in Type 18 are dark green in colour and possess deep red veins on both the sutures. In Type 36, on the other hand, the pods are green in colour but have no red veins on the sutures. The F_1 and the heterozygotes possess green pods with faint red veins on the sutures. It must be mentioned, that soil fertility greatly influences the colour of the pods and very often in poor patches of soil the dark green colour in the pods of Type 18 is lost altogether and only a pale yellow colour is developed on them. In such cases it becomes difficult to differentiate between the three phenotypes for pod colour. The presence or absence of the red veins on the sutures is then the only criterion for differentiation and has, therefore, been a more suitable index than the colour itself in recognizing the different phenotypes for pod colour.

The following phenotypes and frequencies were obtained for flower and pod colours :—

Table II

F_2 segregation for the inheritance of flower and unripe pod

Series	No. of plants with flower and pod colours like			Total No. of plants	χ^2	$P <$
	Type 18	Intermediate	Type 36			
A	31	64	25	120	1·133	0·50
B	28	67	36	131	1·097	0·50
Total .	59	131	61	251		

The values of χ^2 and P shown in Table II indicate that the observed and expected frequencies are in harmony and that there is only a single factor difference between the colour of the flowers or of unripe pods.

Representing the flower colour and the unripe pod colour phenotype, resembling Type 18 parent, by the genes **OO** (Light yellowish olive flowers and the dark green pods having deep red veins on the sutures) and the Type 36 phenotype by the allelomorph **oo** (Olive yellow flowers and green pods having no red colour on the sutures), the mode of segregation for these two characters may be shown as—

1 **OO** : 2 **Oo** : 1 **oo**

This hypothesis is fully confirmed by the behaviour of the next generation as is shown below :—

Sixty-four families of this cross were studied in the F_3 generation. Of these twelve families each bred true for the Type 18 and Type 36 phenotypes respectively and forty families segregated for both the characters. Table III shows the frequencies observed in these segregating families.

The fit between observed and expected frequencies is very close indeed. This is confirmed by Fig. 1 which shows graphically the observed and expected χ^2 distributions for the forty segregating F_3 families.

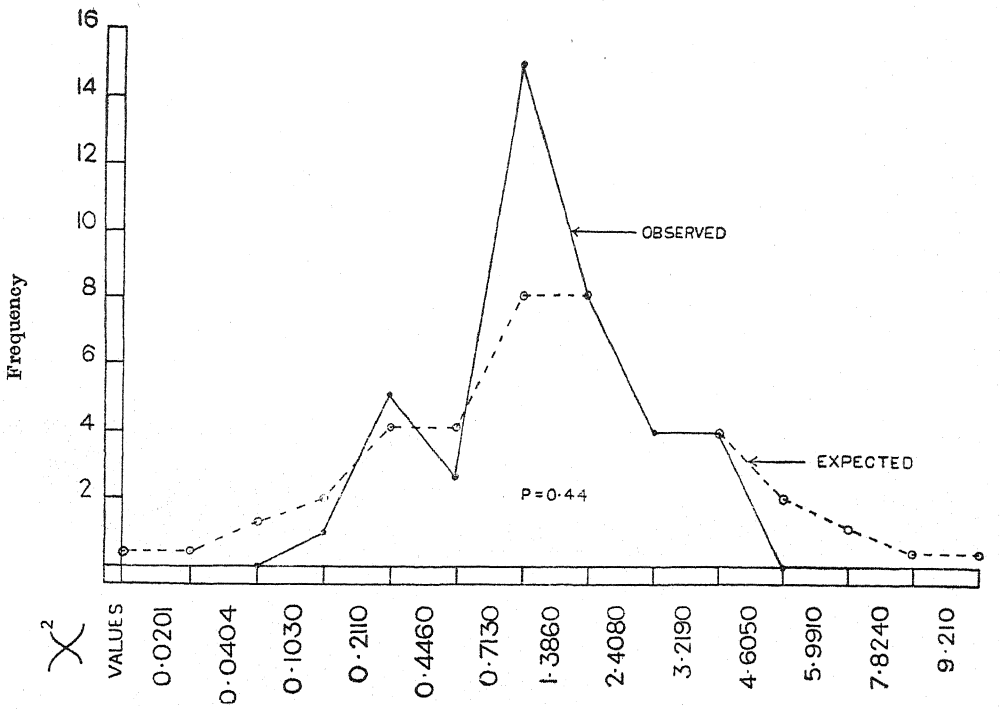


FIG. 1. Observed and expected χ^2 distributions of forty F_3 families segregating for flower and pod colours

It will be observed from Fig. 1 that the deviation between the observed and expected frequencies is insignificant, the value of P being 0·44.

(2) Inheritance of the seed-coat colour and the nature of the seed surface

The seed-coat colour in the *mung* cross under investigation may be considered from the economic view-point as the most important character studied and its inheritance has been found to depend on the interaction of two genes as will be described in the following pages. The dull surface of the seed-coat, on the other hand, has been observed to be dominant to shining seed surface, the F_2 showing a clear-cut monogenic type of segregation for the character.

The inheritance of the seed-coat colour and of the seed surface apparently depend on independent genes and no linkage between the two characters has been noted. Similarly there is no linkage between either of these two characters and flower colour.

(a) Seed-coat colour.—The seeds of Type 18 and those of the F_1 plants were characterised by a dark green colour and a dull surface, while the seeds of the Type 36 parent were of a pale lemon yellow colour and had a shining surface. The F_2 showed the phenotypes and frequencies tabulated below :—

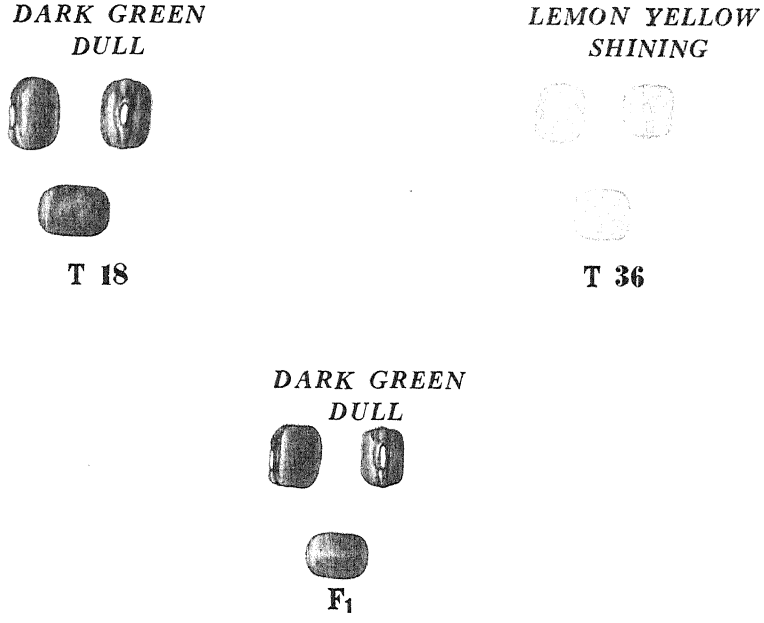
TABLE III

F_2 segregation on a 9 : 3 : 3 : 1 basis for the inheritance of the seed-coat colour

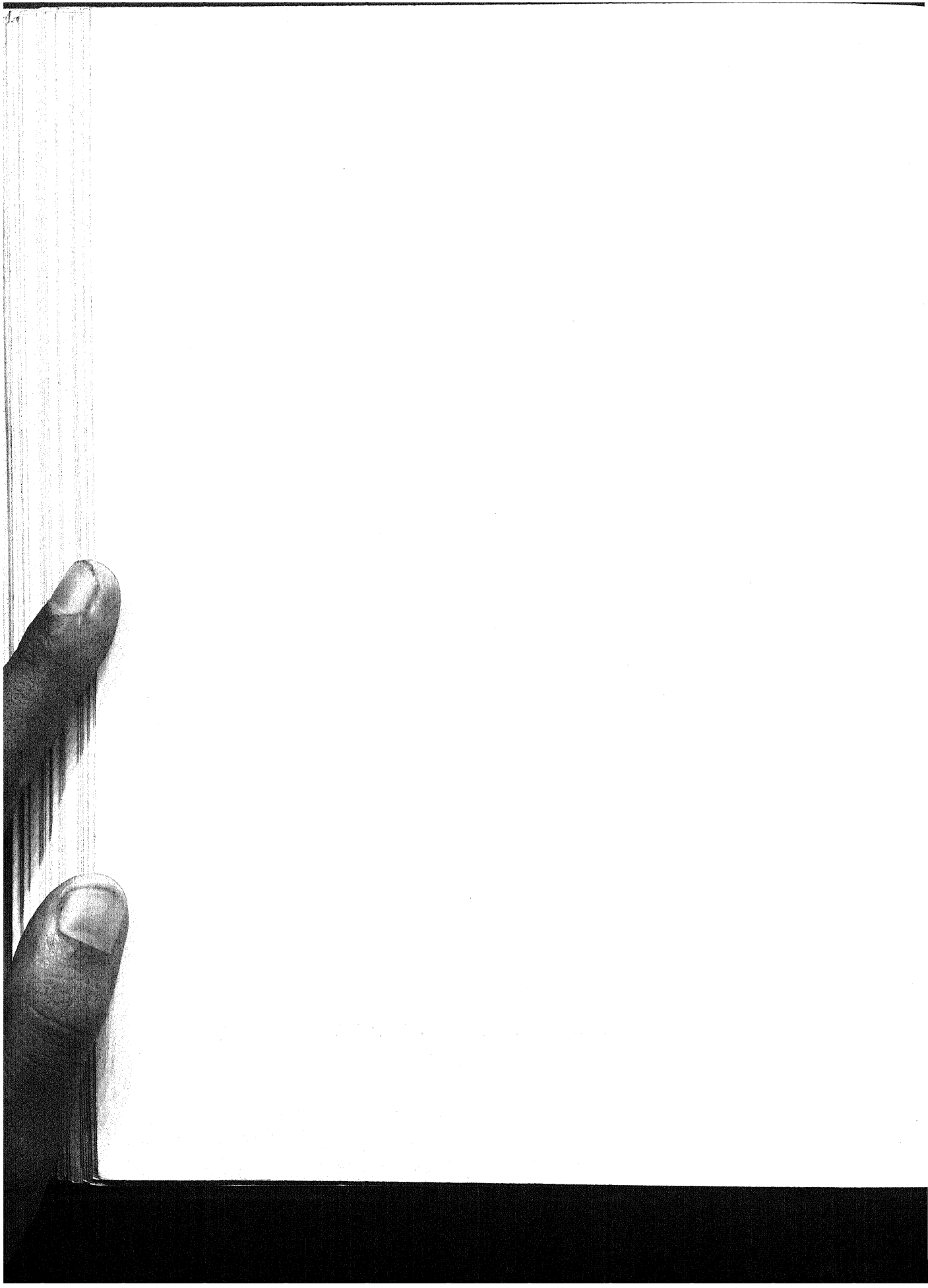
Series	No. of plants with seed colour				Total No. of plants	χ^2	$P <$
	Dark green	Green	Greenish yellow	Pale lemon yellow			
A . .	66	20	24	10	120	1·2440	0·70
B . .	74	25	23	9	131	0·1889	0·95
Total .	140	45	47	19	251		

The fit between observed and expected frequencies is extremely close and shows definitely that two genes are responsible for the inheritance of seed-coat colour in the cross under study. The factorial basis for this may be assumed to be as follows :—

Let **AA** be a pair of genes responsible for the production of green colour in *mung* seeds and let **BB** be a pair of genes which imparts a darker colour to the seed-coat. The genes **AAbb** would, therefore, produce green coloured seeds and **aabb** would give rise to pale yellow seeds (or seeds with green colour absent). The presence of the genes **AABB** together would result in the production of dark green seeds



Inheritance of seed colour in *mung* cross T 18 × T 36



and of the genes **aaBB** would produce greenish yellow seeds. The genic constitution of the Type 18 parent could therefore be represented by **AABB** and that of the Type 36 parent by **aabb**. Four phenotypes were observed in the F_2 in the proportion 9 dark green : 3 green : 3 greenish yellow : 1 pale lemon yellow. This hypothesis was fully substantiated by the results obtained in the F_3 generation.

(*b*) *Nature of the seed surface.*—In respect of the seed surface, the F_2 generation showed the following segregation :—

TABLE IV

F_2 segregation for the nature of the seed surface

Series	Plants with seed surface		Total No. of plants	χ^2	*P*
	Dull	Shining			
A . .	95	25	120	0·5560	0·30
B . .	101	30	131	0·3114	0·50
Total .	196	55	251		

The fit between observed and expected frequencies is close and suggests a single factor difference between dull and shining seeds.

A single factor pair **CC** and **cc** controls the seed surface, and the genic constitution of the parental types in respect of seed colour and seed surface can be represented as follows :—

Type 18 parent . . . **AABBCC**
Type 36 parent . . . **aabbcc**

Considering the seed colour and the seed surface characters together, the segregation in F_2 shows a good fit on the expectation of a 27 : 9 : 9 : 3 : 9 : 3 : 3 : 1 ratio, indicating that the two characters are segregating independently of each other. The actual ratios obtained are presented in Table V given below :—

TABLE V

F_2 segregation on a trihybrid basis for seed-coat colour and the nature of seed surface

Series	F_2 plants with seeds								Total No. of plants	χ^2	*P* <
	Dark green		Green		Greenish-yellow		Pale lemon yellow				
	Dull	Shining	Dull	Shining	Dull	Shining	Dull	Shining			
A .	54	12	14	6	20	4	7	3	120	4·192	0·70
B .	60	14	21	4	15	8	5	4	131	5·853	0·50
Total .	114	26	35	10	35	12	12	7	251		

Even with small F_2 populations of only 120 and 131 plants respectively the fit between observed and expected frequencies on a trihybrid basis is quite close and confirms the fact that there is no linkage between the characters studied. This is further substantiated by the behaviour of the F_3 families.

That the factors for seed-coat colour and flower colour and also for seed surface and flower colour segregate independently of each other is further seen from the segregation shown in Tables VI and VII respectively.

TABLE VI

Relation in F_2 of seed colour to flower colour

Series / Flowers like	No. of plants with seeds: Dark green T 18	Dark green Inter.	Dark green T 36	Green T 18	Green Inter.	Green T 36	Greenish yellow T 18	Greenish yellow Inter.	Greenish yellow T 36	Pale lemon yellow T 18	Pale lemon yellow Inter.	Pale lemon yellow T 36	Total No. of plants	χ^2	$P <$
A	18	34	14	5	12	3	6	12	6	2	6	2	120	2·1	0·99
B	18	39	17	3	13	9	7	9	7	0	6	3	131	7·989	0·70
Total	36	73	31	8	25	12	13	21	13	2	12	5	251		

In spite of the small population clear indications of independent segregation of seed-coat colour on a 9 : 3 : 3 : 1 basis and of flower colour on 1 : 2 : 1 ratio have been obtained.

TABLE VII

Relation in F_2 of the nature of seed surface to flower colour

Series / Flowers like	No. of plants with seed surface: Dull T 18	Dull Inter.	Dull T 36	Shining T 18	Shining Inter.	Shining T 36	Total No. of plants	χ^2	$P <$
A	24	52	19	7	12	6	120	1·498	0·90
B	21	50	30	7	17	6	131	2·213	0·80
Total	45	102	49	14	29	12	251		

Here again it is quite evident that there is no linkage between the seed surface character and flower colour. As the colour of the unripe pod is completely linked with the colour of the flower in this cross, it is obvious that there is no linkage between the colour of the unripe pod and seed colour as well as with seed surface.

This conclusion is supported by the behaviour of the sixty-four families which were studied in detail in the F_3 generation, the full details of which are given in Table VIII.

TABLE VIII

F_3 frequencies for the inheritance of seed colour and the nature of the seed surface as well as of the colour of the flower and of the unripe pods in the same families —mung *Type 18 × Type 36 (1935)*

Family	Seed colour and seed surface													Flower and unripe pod colours					
	F_1 phenotype	F_3 genotype	No. of plants with seed colour and seed surface								Total No. of plants	χ^2	$P <$	No. of plants with flower colour genotype like			Total No. of plants	χ^2	$P <$
			Dark green		Green		Greenish yellow		Pale lemon yellow										
			Dull	Shining	Dull	Shining	Dull	Shining	Dull	Shining				Type 18	Intermediate	Type 36			
B111	Dark green, dull	AABBCC	All	...	...	...	...	...	...	...	All	...	...	All	...	...	All	...	...
B126	,, ,, ,,	,,	All	...	...	...	...	...	...	...	All	...	...	...	...	All	All	...	...
B40	,, ,, ,,	,,	All	...	...	...	...	...	...	...	All	...	...	19	48	20	87	0·954	0·50
B125	,, ,, ,,	,,	All	...	...	...	...	...	...	...	All	...	...	24	53	18	95	2·031	0·30
B115	,, ,, ,,	AABBCc	72	25	...	...	...	...	...	...	97	0·031	0·80	All	...	...	All	...	...
B83	,, ,, ,,	,,	70	16	...	...	...	...	...	...	86	1·875	0·10	...	...	All	All	...	...
B38	,, ,, ,,	AABbCC	70	...	24	...	...	...	...	...	94	0·014	0·80	21	54	19	94	2·170	0·30
B51	,, ,, ,,	AaBBCC	79	...	...	...	26	...	...	...	105	0·003	0·96	19	55	28	102	2·216	0·30
B108	,, ,, ,,	,,	59	...	...	...	17	...	...	...	76	0·281	0·50	19	33	23	75	1·505	0·30
B76	,, ,, ,,	AABbCc	47	20	17	8	...	...	...	...	92	1·947	0·50	All	...	...	All	...	...
B19	,, ,, ,,	,,	61	19	21	11	...	...	...	...	112	2·537	0·30	32	53	25	110	1·036	0·50
B22	,, ,, ,,	,,	56	19	14	4	...	...	...	...	93	1·643	0·50	27	46	30	103	1·349	0·50
B29	,, ,, ,,	,,	48	20	25	8	...	...	...	...	101	3·819	0·20	33	49	18	100	4·540	0·10
B31	,, ,, ,,	,,	39	18	21	6	...	...	...	...	84	3·617	0·30	28	36	20	84	3·238	0·10
B55	,, ,, ,,	,,	61	24	24	11	...	...	...	...	120	2·458	0·30	32	55	33	120	0·850	0·50
B49	,, ,, ,,	AaBBCc	36	10	...	...	9	4	...	...	59	1·506	0·50	All	...	...	All	...	...

TABLE VIII—*contd.*

Family	F_2 phenotype	F_3 genotype	Seed colour and seed surface: No. of plants with seed colour and seed surface								Total No. of plants	χ^2	$P <$	Flower and unripe pod colours: No. of plants with flower colour genotype like			Total No. of plants	χ^2	$P <$
			Dark green		Green		greenish yellow		Pale lemon yellow										
			Dull	Shining	Dull	Shining	Dull	Shining	Dull	Shining				Type 18	Intermediate	Type 36			
B114	Dark green, dull	AaBBCc	50	24	...	...	15	6	...	...	95	2·812	0·30	All	...	...	All	...	...
B110	,, ,, ,,	,,	40	17	...	...	20	5	...	...	82	2·340	0·50	22	40	20	82	0·146	0·90
B79	,, ,, ,,	AaBbCC	46	...	13	...	17	...	4	...	80	0·752	0·80	25	38	17	80	1·800	0·30
B97	,, ,, ,,	AaBbCc	34	14	13	4	10	3	4	2	84	1·430	0·98	All	...	...	All	...	...
B25	,, ,, ,,	,,	38	14	12	5	8	3	4	2	86	2·520	0·90	...	...	All	All	...	...
B2	,, ,, ,,	,,	45	15	20	6	18	6	8	3	121	3·140	0·80	33	59	29	121	0·039	0·80
B3	,, ,, ,,	,,	39	9	12	6	8	4	2	4	84	3·680	0·80	24	39	21	84	0·643	0·70
B5	,, ,, ,,	,,	46	11	20	8	12	5	8	3	113	7·190	0·30	23	57	29	109	0·990	0·50
B8	,, ,, ,,	,,	21	6	13	3	12	4	5	1	65	6·610	0·30	20	29	16	65	1·246	0·50
B48	,, ,, ,,	,,	31	12	14	7	15	4	6	3	92	6·070	0·50	23	41	25	89	0·640	0·70
B50	,, ,, ,,	,,	28	14	15	8	12	4	9	6	96	16·070	0·02	27	48	22	97	0·526	0·70
B75	,, ,, ,,	,,	31	15	19	6	11	1	7	3	93	11·450	0·10	20	50	17	87	2·149	0·30
B80	,, ,, ,,	,,	31	16	10	5	6	3	5	2	78	6·330	0·50	16	36	26	78	3·025	0·20
B92	,, ,, ,,	,,	38	9	14	8	5	4	8	4	90	17·380	0·02	26	41	21	88	0·977	0·50
B32	Dark green, shining	AABBcc	...	All	...	...	...	...	...	...	All	...	...	All	...	...	All	...	...
B34	,, ,, ,,	,,	...	All	...	...	...	...	...	...	All	...	...	All	...	...	All	...	...
B91	,, ,, ,,	AABbcc	...	72	...	31	...	...	...	...	103	1·424	0·20	All	...	...	All	...	...
B73	,, ,, ,,	,,	...	42	...	20	...	...	...	...	62	1·740	0·10	13	26	21	60	3·199	0·20
B89	,, ,, ,,	,,	...	45	...	17	...	...	...	...	62	0·193	0·70	19	28	15	62	1·097	0·50
B90	,, ,, ,,	,,	...	51	...	23	...	...	...	...	74	1·456	0·20	23	27	16	66	3·666	0·10

B119	„ „ „	AaBBcc	...	75	...	...	...	26	...	...	101	0·028	0·80	27	51	23	101	0·327	0·80
B63	„ „ „	AaBbcc	...	45	...	14	...	18	...	8	85	2·027	0·70	21	49	15	85	2·735	0·20
B130	Green, dull	AAbbCC	...	...	All	...	...	...	...	...	All	...	...	22	34	16	72	1·222	0·50
B98	„ „	AAbbCc	...	...	75	31	...	...	...	...	106	1·016	0·30	...	...	All	All	...	...
B105	„ „	„	...	...	71	26	...	...	...	...	97	0·168	0·70	...	...	All	All	...	...
B7	„ „	„	...	...	85	25	...	...	...	...	110	0·300	0·50	23	57	29	109	0·900	0·50
B13	„ „	AabbCC	...	...	49	...	...	...	15	...	64	0·080	0·70	All	...	...	All	...	...
B56	„ „	„	...	...	69	...	...	...	18	...	87	0·870	0·30	...	...	All	All	...	...
B16	„ „	AabbCc	...	...	43	19	...	...	14	3	79	3·771	0·20	17	39	23	79	0·924	0·50
...	Green, shining	AAbbcc	(No families realised)																
B45	„ „	Aabbcc	...	...	...	46	...	...	...	22	68	1·960	0·10	...	...	All	All	...	...
B72	„ „	„	...	...	...	74	...	...	...	27	101	0·160	0·50	28	48	25	101	0·428	0·80
B131	„ „	„	...	...	...	69	...	...	...	19	88	0·545	0·30	23	36	14	73	1·120	0·50
B70	Greenish yellow, dull	aaBBCC	...	...	...	...	All	...	...	...	All	...	...	All	...	...	All	...	...
B54	„ „	aaBBCc	...	...	...	...	47	17	...	...	64	0·080	0·70	All	...	...	All	...	...
B71	„ „	„	...	...	...	...	74	19	...	...	93	1·030	0·30	21	52	20	93	1·322	0·50
B122	„ „	aaBbCC	...	...	...	...	55	...	26	...	81	2·176	0·10	...	...	All	All	...	...
B21	„ „	aaBbCc	...	...	...	...	59	17	24	9	109	1·979	0·10	28	56	25	109	0·247	0·80
B30	„ „	„	...	...	...	...	49	14	15	4	82	0·556	0·30	23	35	24	82	1·780	0·30
B121	„ „	„	...	...	...	...	44	17	18	4	83	0·534	0·30	19	46	18	83	1·000	0·50
B128	Greenish yellow, shining	aaBBcc	...	...	...	...	...	All	...	...	All	...	...	...	...	All	All	...	...
B74	„ „ „	aaBbcc	...	...	...	...	...	29	...	8	37	0·220	0·50	13	21	17	41	1·750	0·30
B112	„ „ „	„	...	...	...	...	...	62	...	20	82	0·016	0·90	27	40	14	81	4·185	0·10
...	Pale lemon yellow, dull	aabbCC	(No families realised.)																
B127	„ „ „	aabbCc	...	...	...	...	...	...	66	18	84	0·570	0·30	...	...	All	All	...	...
B116	„ „ „	„	...	...	...	...	...	...	72	17	89	1·640	0·20	26	39	21	86	1·325	0·50
B28	Pale lemon yellow, shining	aabbcc	...	...	...	...	...	...	...	All	All	...	...	...	...	All	All	...	...
B61	„ „ „	„	...	...	...	...	...	...	...	All	All	...	...	...	...	All	All	...	...
B37	„ „ „	„	...	...	...	...	...	...	...	All	All	...	...	22	56	19	97	2·505	0·20
B43	„ „ „	„	...	...	...	...	...	...	...	All	All	...	...	18	30	15	63	0·428	0·80

The behaviour of the sixty-four F_3 families for the qualitative characters studied is clearly brought out in Table VIII. The genotypic results are in harmony with the expectations and prove that the inheritance of the seed-coat colour in this cross is dependent on the interaction of two genes, while that of seed surface and flower colour are monogenic in nature. Each of these characters is also inherited independently.

It is evident from the χ^2 values provided in the table that most of the individual families do not show any significant departure from the expected distribution.

In the segregation for seed colour it is found that the ' dark green ' group is somewhat, though not significantly, deficient in some families. This is possibly due to the fact that the heterozygote is slightly less pigmented than the homozygote and in studying the segregation of factor **B** a few of these have been wrongly included in the recessive class. It may be pointed out that families with the genotypes **AAbbcc** and **aabbCC** were not realised in the F_3, but have since been isolated from the heterozygotes of these groups.

The genetic constitution of the two parental types for the qualitative characters studied may, therefore, be represented as follows :—

Type 18 parent . . . . **AABBCCOO**
Type 36 parent . . . . **aabbccoo**

IV. Inheritance of quantitative characters

The environment usually brings in many complexities in the way of the elucidation of the mode of inheritance of quantitative characters, but given approximately similar sets of soil, cultural and climatic conditions to parental as well as to other filial generations, it is usually possible to obtain reliable indications regarding the inheritance of such characters.

In the studies reported herein the parental types were always grown along with the F_1, F_2 and F_3 generations and the differences in the range of distribution of plants in the parental types for a particular character, in different years, furnished a basis for determining the seasonal effect therein. Thus it will be seen from Table XI that the average diameter of pods in 1933 was definitely lower in both the parents than those observed in the two subsequent years. Hence the diameter of pods observed in the F_1 plants raised in 1933 cannot be compared directly with those observed in the F_2 and F_3 populations grown in 1934 and 1935 respectively. The effect of the season cannot, therefore, be neglected in comparing average values of a particular quantitative character in studies such as these.

(1) *Inheritance of maturity as measured by the date of the appearance of the first flower*

Owing to the incidence of desiccating west winds at the time when the *mung* crop comes to maturity, it is not possible to appreciate differences in the maturity of different plants and hence the date of the appearance of the first flower in the plant has been considered to furnish a more reliable index of maturity than the actual date of ripening. The frequency distributions of the parental, F_1, F_2 and F_3 families are presented in Table IX.

TABLE IX

Frequency distribution of the number of days taken to flower in mung

Family	Year	Class centres of No. of days taken to flower												Total No. of plants	Mean
		55	58	61	64	67	70	73	76	79	82	85	88		
Type 18 parent . .	1933	...	...	...	1	3	6	35	16	2	3	...	...	66	73·64
Type 18 parent . .	1934	...	...	...	...	2	6	25	11	2	...	...	...	46	73·31
Type 18 parent . .	1935	...	...	...	...	2	35	50	23	12	1	...	...	123	73·28
Type 36 parent . .	1933	1	3	9	38	17	6	2	...	...	...	...	...	76	64·67
Type 36 parent . .	1934	...	1	15	19	14	1	...	...	...	...	...	...	50	63·94
Type 36 parent . .	1935	1	5	70	28	15	3	...	...	...	...	...	...	122	62·48
Type 18 × Type 36, F_1 .	1933	2	...	...	...	...	...	...	...	...	...	...	...	2	56·00
Type 18 × Type 36, F_2 (Series A).	1934	...	1	4	32	27	29	8	12	6	1	...	...	120	67·67
Type 18 × Type 36, F_2 (Series B).	1934	...	1	6	40	32	28	11	11	2	...	...	...	131	67·83
Type 18 × Type 36, F_3 —															
B 115 . . .	1935	1	30	58	11	1	1	...	...	...	...	...	...	102	60·53
B 116 . . .	1935	...	5	39	44	5	...	...	...	...	...	...	...	93	62·58
B 13 . . .	1935	...	3	32	23	7	...	...	...	...	...	...	...	65	62·57
B 22 . . .	1935	...	...	...	1	20	57	25	4	1	...	...	...	108	70·39
B 29 . . .	1935	...	...	...	1	37	52	7	3	1	...	...	...	101	69·32
B 122 . . .	1935	...	...	...	1	6	30	41	7	4	...	...	...	89	71·99
B 125 . . .	1935	...	...	...	...	1	20	43	20	15	1	...	1	101	74·07
B 126 . . .	1935	...	1	17	39	37	10	5	4	2	1	1	...	117	66·36
B 34 . . .	1935	...	1	3	2	35	42	15	2	1	2	...	...	103	69·36
B 56 . . .	1935	3	8	9	21	45	20	10	6	5	4	1	...	132	67·66
B 63 . . .	1935	...	1	9	17	34	32	15	2	1	3	2	...	116	68·58
B 83 . . .	1935	...	...	1	19	28	30	10	5	3	1	1	...	98	69·05

It is found that the range of distribution in Type 18 extends from sixty four to eighty-two days approximately with a mean of 73·6 days and in Type 36 from fifty-five to seventy-three days with a mean of about 64·6 days The seasonal variation does not appear to influence this character very much. The F_1 plants flowered in fifty-six days showing a definite dominance of earliness. This supports Fisher's theory that a character for which there has been selection for a long time always shows dominance. The range of the F_2 exceeds the combined range of the two parents and definitely suggests transgressive segregation. The distribution of only a dozen homozygous and heterozygous F_3 families has been shown in the table but this character was studied in sixty-four families and definitely indicated that multiple factors were concerned in the inheritance of maturity in the cross under study.

(2) *Inheritance of the length of ripe pods*

Although the average length of pods in the two parents differs but slightly and the F_1 plants showed a tendency to have pods of a similar size to those of Type 36, the range of the F_2 progenies showed a clear one-sided transgression towards the smaller pod length. This fact is shown in Table X. The data from the F_3 families also confirmed the conclusion that multiple factors were responsible for the inheritance of this character.

(3) *Inheritance of the diameter of pods*

The frequency distributions of the parental and F_2 and F_3 families are presented in Table XI. Multiple factors appear to be responsible for the inheritance of this character also but the transgression is rather one-sided and the frequency of plants with greater diameters than the parental forms is distinctly greater. It is possible that heterosis is responsible for the nature of the transgression shown.

TABLE X

Frequency distributions of the length of ripe pods in mung

Family	Year	Class centres of length of pods in cms.													Total No. of plants	Mean
		F_2 parent	3·0	3·5	4·0	4·5	5·0	5·5	6·0	6·5	7·0	7·5	8·0	8·5	9·0		
Type 18 parent	1933	...	...	...	...	...	...	4	22	23	11	...	1	...	...	60	6·342
Type 18 parent	1934	...	...	...	...	...	4	9	26	28	25	8	...	...	...	100	6·425
Type 18 parent	1935	...	...	...	...	...	...	9	28	31	29	5	...	...	...	102	6·466
Type 36 parent	1933	...	...	...	...	...	...	...	1	3	19	28	9	...	...	60	7·342
Type 36 parent	1934	...	...	...	...	...	...	1	7	18	41	25	3	...	...	95	6·979
Type 36 parent	1935	...	...	...	...	...	...	5	16	19	31	30	8	...	...	109	6·908
Type 18 × Type 36, F_1	1933	...	...	...	...	...	...	...	...	...	2	...	...	...	...	2	7·000
Type 18 × Type 36, F_2 (Series A)	1934	...	...	...	2	3	3	10	21	28	37	14	2	...	...	120	6·496
Type 18 × Type 36, F_2 (Series B)	1934	...	...	1	3	2	8	10	18	41	35	10	3	...	...	131	6·397
Type 18 × Type 36, F_3—																	
B 22	1935	...	...	4	6	4	11	31	37	9	...	...	...	...	...	102	5·510
B 83	1935	...	...	...	...	2	7	18	32	18	1	...	...	...	...	78	5·885
B 115	1935	...	...	...	...	5	11	19	25	33	2	...	...	...	...	95	5·900
B 116	1935	...	...	...	...	6	5	11	24	14	18	1	...	...	...	79	6·089
B 126	1935	...	...	...	...	1	4	7	28	37	18	2	...	...	...	97	6·314
B 125	1935	...	...	...	...	...	...	13	28	29	26	3	...	...	...	99	6·389
B 56	1935	...	...	...	...	...	4	8	11	33	25	1	...	...	...	82	6·427
B 29	1935	...	2	1	2	3	7	23	27	18	12	4	1	...	...	100	5·935
B 13	1935	...	...	...	1	3	6	12	18	12	5	2	1	...	...	60	5·958
B 122	1935	...	...	1	1	1	4	4	12	21	19	12	3	...	...	78	6·532
B 34	1935	...	...	1	3	5	5	13	30	22	14	1	2	...	...	96	6·031
B 63	1935	..	1	1	1	2	4	7	16	17	22	2	1	2	1	77	6·331

TABLE XI

Frequency distributions of the diameter of ripe pods in mung

Family	Year	Class centres of diameter of pods in cms.										Total No. of plants	Mean
		F_2 parent	0·25	0·28	0·31	0·34	0·37	0·40	0·43	0·46	0·49	0·52	
Type 18 parent . .	1933	...	...	7	28	22	3	...	...	...	...	...	60	0·321
Type 18 parent . .	1934	...	...	...	6	24	60	9	1	...	...	...	100	0·363
Type 18 parent . .	1935	...	...	...	2	36	40	21	3	...	...	...	102	0·366
Type 36 parent . .	1933	...	...	...	12	22	24	2	...	...	...	...	60	0·348
Type 36 parent . .	1934	...	...	...	...	3	13	69	10	...	...	...	95	0·397
Type 36 parent . .	1935	...	...	...	8	10	72	18	1	...	...	...	109	0·368
Type 18 × Type 36—F_1 .	1933	...	...	...	...	...	2	...	...	...	...	...	2	0·370
Type 18 × Type 36—F_2 (Series A)	1934	...	...	1	1	3	12	34	48	20	1	...	120	0·411
Type 18 × Type 36—F_2 (Series B)	1934	...	...	1	2	2	9	23	58	33	2	1	131	0·425
Type 18 × Type 36—F_3—														
B 30	1935	0·37	2	3	15	27	24	2	1	...	...	...	74	0·34
B 130	1935	0·38	...	1	2	5	21	26	18	6	2	2	83	0·40
B 28	1935	0·39	...	2	6	8	30	10	4	...	...	...	60	0·37
B 89	1935	0·40	...	...	...	2	10	22	20	9	2	...	65	0·40
B 5	1935	0·40	...	...	3	20	45	37	5	2	...	...	112	0·38
B 2	1935	0·40	...	...	4	13	35	21	28	2	...	...	103	0·39
B 127	1935	0·40	...	...	2	10	41	24	6	...	...	...	83	0·38
B 116	1935	0·41	...	...	3	5	37	25	7	2	...	...	79	0·38
B 74	1935	0·41	...	...	1	3	19	7	4	...	...	...	34	0·38
B 34 . . .	1935	0·42	...	...	...	3	16	31	35	8	3	...	96	0·41
B 108	1935	0·42	...	...	...	5	10	18	25	16	3	...	77	0·42
B 114	1935	0·42	...	...	...	1	16	39	32	8	1	...	97	0·41

B 119	1935	0·42	...	...	1	11	22	42	18	2	...	...	96	0·39
B 25	1935	0·42	...	1	2	5	13	16	23	14	4	...	78	0·41
B 48	1935	0·42	...	...	...	7	24	25	24	6	2	...	88	0·41
B 49	1935	0·42	...	1	2	5	13	26	4	...	...	...	51	0·38
B 91	1935	0·42	...	...	...	3	14	26	33	21	2	...	99	0·42
B 29	1935	0·43	...	...	6	11	34	29	19	1	...	...	100	0·38
B 56	1935	0·43	...	...	...	3	9	35	31	4	...	...	82	0·41
B 126	1935	0·43	...	1	2	3	14	25	36	14	2	...	97	0·41
B 21	1935	0·43	...	...	9	20	31	34	5	...	...	...	99	0·38
B 54	1935	0·43	...	...	...	5	11	28	15	2	...	...	61	0·40
B 73	1935	0·43	...	...	...	4	4	12	22	15	6	...	63	0·43
B 76	1935	0·43	...	...	...	8	16	26	25	13	1	...	89	0·41
B 31	1935	0·43	...	...	1	7	22	30	22	3	1	1	87	0·40
B 45	1935	0·43	...	1	3	7	16	26	9	3	1	...	66	0·39
B 79	1935	0·43	...	2	5	12	27	16	9	...	...	...	71	0·37
B 80	1935	0·43	1	2	5	6	12	26	18	6	1	...	77	0·39
B 122	1935	0·44	...	...	...	3	14	16	31	12	2	...	78	0·42
B 83	1935	0·44	...	...	1	9	23	36	7	2	...	...	78	0·39
B 32	1935	0·44	1	2	4	10	24	29	17	6	...	...	93	0·39
B 37	1935	0·44	...	2	7	12	34	35	16	1	...	...	107	0·38
B 43	1935	0·44	...	1	5	8	17	24	6	1	...	...	62	0·38
B 3	1935	0·44	...	...	...	4	17	26	33	3	...	...	83	0·41
B 7	1935	0·44	...	...	2	6	41	45	12	...	...	...	106	0·39
B 16	1935	0·44	...	...	2	1	5	12	26	24	3	...	73	0·43
B 40	1935	0·44	...	...	...	6	13	28	29	2	1	...	79	0·40
B 51	1935	0·44	...	...	5	8	27	25	29	7	...	...	101	0·40
B 22	1935	0·45	...	2	3	14	35	38	8	1	...	...	101	0·38
B 125	1935	0·45	...	...	2	5	16	32	42	3	...	...	100	0·40
B 63	1935	0·45	...	...	4	4	17	24	13	13	2	...	77	0·43
B 115	1935	0·45	...	...	...	5	24	34	26	6	...	...	95	0·41

TABLE XI—*contd.*

Family	Year	Class centres of diamater of pods in cms.										Total No. of plants	Mean
		F_2 parent	0·25	0·28	0·31	0·34	0·37	0·40	0·43	0·46	0·49	0·52		
B 131	1935	0·45	...	...	...	1	12	22	38	6	1	...	80	0·41
B 97	1935	0·45	...	...	2	12	22	21	16	1	...	...	74	0·39
B 72	1935	0·45	...	2	3	4	18	21	34	14	1	...	97	0·40
B 111	1935	0·45	...	...	...	3	6	12	19	12	1	...	53	0·42
B 112	1935	0·45	...	...	1	3	11	18	28	10	1	...	72	0·41
B 8	1935	0·45	...	...	2	2	18	19	19	3	2	...	65	0·40
B 92	1935	0·45	...	...	2	6	16	28	27	6	1	...	86	0·40
B 50	1935	0·46	...	...	...	2	12	26	23	24	1	...	88	0·42
B 70	1935	0·46	...	...	3	10	38	26	22	3	...	...	102	0·39
B 71	1935	0·46	1	2	10	19	21	15	14	2	...	...	84	0·37
B 98	1935	0·46	...	...	...	9	24	22	34	13	...	...	102	0·40
B 121	1935	0·46	1	1	3	16	34	20	7	2	1	...	85	0·38
B 55	1935	0·46	1	3	11	38	38	21	4	2	1	...	119	0·36
B 61	1935	0·46	2	2	8	16	28	35	6	3	2	...	102	0·38
B 99	1935	0·46	...	...	2	6	12	24	18	3	...	...	65	0·39
B 105	1935	0·46	...	1	3	4	18	30	23	14	2	1	96	0·41
B 13	1935	0·47	...	...	...	1	16	17	17	9	...	...	60	0·40
B 38	1935	0·47	1	...	3	6	14	30	28	6	1	...	89	0·40
B 19	1935	0·47	...	1	1	1	7	22	35	34	6	1	108	0·43
B 110	1935	0·48	...	...	1	6	6	22	21	7	3	...	66	0·38
B 75	1935	0·51	...	...	...	3	26	28	20	9	3	...	89	0·40

The frequency distributions of all F_3 families studied for this character have been shown in this table and have been arranged according to the size of the diameter of the pods of the F_2 parents. It will be found that F_2 plants having the same diameter have produced F_3 families which are homozygous as well as heterozygous for the diameter of their pods.

V. Inter-relationship of the characters studied

The colour of the unripe pod has been found to be completely linked with flower colour, and a positive correlation ($r=0\cdot50\pm0\cdot03$) has been found to exist between the length and diameter of ripe pods in this cross, but there is no evidence of any association existing between :--

(1) Maturity and length of pods,
(2) Maturity and diameter of pods,
(3) Maturity and flower colour,
(4) Maturity and seed colour,
(5) Maturity and seed surface,
(6) Length of pod and flower colour,
(7) Length of pod and seed colour,
(8) Length of pod and seed surface,
(9) Diameter of pod and flower colour,
(10) Diameter of pod and seed colour, and
(11) Diameter of pod and seed surface.

An example to show the absence of any relationship between a qualitative and a quantitative character is furnished in Table XII given below :—

Table XII

Relation between maturity and flower colour in F_2

Flower colour like.	No. of days taken by the first flower to appear									Total No. of plants	Mean	S. Dev.
	58	61	64	67	70	73	76	79	82			
Type 18 . .	..	3	15	12	17	3	8	1	..	59	68·53 ±0·58	4·43 ±0·41
Intermediate .	2	6	37	32	26	10	12	5	1	131	68·19 ±0·42	4·81 ±0·30
Type 36 . .	..	1	20	15	14	6	3	2	..	61	68·03 ±0·52	4·10 ±0·37
Total .	2	10	72	59	57	19	23	8	1	251	68·23 ±0·29	4·57 ±0·20

There are no significant differences between the means and standard deviations of the different phenotypes for flower colour, indicating that there is no linkage between the two characters.

VI. Summary and conclusions

Genetical studies in some species of *Phaseolus* such as *Ph. vulgaris*, *Ph. chrysanthos*, *Ph. multiflorus*, etc., have been made by workers abroad but similar studies in *mung* or *Phaseolus radiatus* Linn (syn. *P. aureus* Roxb.) do not appear to have been reported up to now. The mode of inheritance of the colour in the flower, unripe pod, and seed-coat and in the nature of the seed-surface, as well as of quantitative characters such as maturity, length and diameter of pods, and the relation of these characters to each other have been studied at Pusa and described in this paper.

A cross was made between Pusa Type 18 and Type 36; the former has dark green, dull seeds, and light yellowish olive flowers while the latter has pale lemon-yellow coloured seeds with a shining surface and olive yellow flowers. Monogenic inheritance has been observed in this cross for flower colour and the nature of the seed surface, olive yellow flowers and shining seed surface (both like those present in Type 36 parent) being recessive. Two genes appear to be responsible for the inheritance of the seed-coat colour.

Transgressive segregation has been noted in respect of the maturity as well as in the length and diameter of the pods. The colour of the unripe pods appears to be controlled by the same genes which are responsible for the inheritance of the flower colour but there is no linkage between seed-coat colour and the nature of the seed surface nor between any of these two characters and flower colour. Neither is there any relationship between any of these qualitative characters with the quantitative characters studied.

A correlation of $r = 0{\cdot}50 \pm 0.03$ has been observed between the length and diameter of pods but no association exists between these two characters and maturity.

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STUDIES ON THE ROOT-ROT DISEASE OF COTTON IN THE PUNJAB

VII. FURTHER INVESTIGATION OF FACTORS INFLUENCING INCIDENCE OF THE DISEASE

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(With two text-figures)

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INTRODUCTORY

COTTON root-rot is the most destructive disease of this crop in the Punjab ; in the irrigated tracts of the Province it is becoming a limiting factor in cotton growing. The causal organisms *Rhizoctonia bataticola** and *Rhizoctonia solani* attack the underground parts of the plants.

In a previous publication of this series [Vasudeva, 1935], the extent of occurrence, incidence and economic importance of the cotton root-rot disease in the Punjab were described. Since then, further studies of the disease have shown that the activity of the causal fungi is greatly influenced by enviros-mental factors [Vasudeva, 1937]. The work reported in this paper relaten to the investigation of any possible connection of temperature, humidity and physical and chemical composition of the soil with the disease.

**Rhizoctonia bataticola* = C Strains of Haigh = *Macrophomina phaseoli.*

II. Experimental

The experiments reported in the present paper were conducted in plots which had been under observation for a number of years. The extent of infection of these plots is definitely known, so that the conditions highly suitable for obtaining reliable and comparable data were available.

A. Effect of soil temperature on the incidence of the disease

To determine the effect of soil temperature on the root-rot disease, inoculation experiments were carried out in soil temperature tanks of Wisconsin type [Jones *et al.*, 1926]. The desired temperature was obtained and maintained with reasonable accuracy during the experimental period by regulation of temperature of water in the tanks by means of electric thermostats. Fairly deep metallic containers were used so that the level of the soil may be kept slightly below that of water in the tank. The containers used were cylindrical and of 9½ inches depth so that it was possible to carry cotton plants for a fairly long period. The tanks were run at 25°, 30°, 35° and 40°C. Cotton plants (*Gossypium hirsutum*) of variety 43F, of known age and growing in these containers, were inoculated by root washing method [Vasudeva, 1935] with the causal fungi *Rhizoctonia solani* and *Rhizoctonia bataticola* and placed in the tanks at the temperatures stated above. The controls were similarly treated except that no inoculum was added. The results of three such experiments are given in Table I. The age of the plants in experiments I, II and III was 16, 24 and 30 days, respectively.

Table I

Effect of soil temperature on infection

Temperature (°C.)	Fungus *	Experiment I			Experiment II			Experiment III		
		Total plants	Plants killed	Per cent mortality	Total plants	Plants killed	Per cent mortality	Total plants	Plants killed	Per cent mortality
25	*R. s.*	11	4	36.4	6	2	33.3	7	3	42.9
	R. b.	12	10	83.3	9	8	88.9	6	3	50.0
	Control	12	0	0	10	0	0	6	0	0
30	*R. s.*	11	5	45.5	10	4	40.0	9	8	88.9
	R. b.	8	5	62.5	9	8	88.9	9	6	66.7
	Control	11	0	0	14	0	0	14	0	0
35	*R. s.*	16	14	87.5	8	5	62.5	7	7	100.0
	R. b.	12	10	83.3	13	12	92.3	6	6	100.0
	Control	14	0	0	7	0	0	2	0	0
39	*R. s.*	10	5	50.0	9	2	22.2	10	1	10.0
	R. b.	14	13	92.9	12	12	100.0	5	5	100.0
	Control	12	0	0	9	0	0	7	0	0

* *R. s.* = *Rhizoctonia solani*, *R. b.* = *Rhizoctonia bataticola*.

The results given in Table I show that cotton plants suffer highest mortality from root-rot due to *R. solani* and *R. bataticola* at temperatures of 35° and 39°C. respectively. Although *R. bataticola* is actively parasitic over a range of temperatures varying from 25° to 39°C., there is a fall noticeable in the parasitic activity of *R. solani* above and below 35°C.

In a similar experiment the effect of variation of temperature after inoculation was studied. The inoculated plants were placed in hot water tanks at soil temperatures of 20° and 35°C. The inoculated containers were allowed to stand in these tanks for twenty-four hours after which two pots inoculated with each of the fungi, *i.e., R. solani and R. bataticola*, were transferred from 35° to 20°C. and from 20° to 35°C. An uninoculated control was also transferred simultaneously. Also inoculated and uninoculated plants were kept as checks constantly at 35°C. and 20°C. in order to compare the effect of variation of temperature on infection. The results of one of the typical experiments are summarised in Table II.

TABLE II

Effect of variation of temperature 24 hours after inoculation on the incidence of the disease

Temperature (°C.)		Pot No.		Fungus	Plants inoculated	Total plants killed	Per cent mortality
Before transfer	After transfer	Transferred	Not transferred				
20	20	—	3	*R. bataticola* .	12	9	75·0
20	35	4 & 5	—	*R. bataticola* .	23	19	82·6
20	35	6 & 7	—	*R. solani* . .	25	25	100·0
20	20	—	8	*R. solani* . .	9	1	11·1
20	35	2	—	Control . .	10	0	0 0
20	20	—	1	Control . .	14	0	0·0
35	20	11 & 12	—	*R. bataticola* .	18	15	83·3
35	35	—	13	*R. bataticola* .	10	9	90·0
35	20	14 & 16	—	*R. solani* . .	21	4	19·0
35	35	—	15	*R. solani* . .	13	13	100·0
35	20	10	—	Control . .	13	0	0·0
35	35	—	9	Control . .	13	0	0·0

The results show that in the case of *R. solani* if the temperature is lowered twenty-four hours after inoculation the incidence of the disease is definitely reduced but in the case of *R. bataticola* such a fall in temperature does not materially affect the incidence of the disease.

However when temperature is raised after inoculation from 20° to 35°C. the mortality in the case of both the fungi is almost as high as in controls maintained at 35°C. throughout.

B. Effect of humidity and temperature on the incidence of the disease

A heavily infected plot was selected in the Botanical area and divided into six sub-plots. *Desi* cotton (*Gossypium indicum*) variety Mollisoni 39 was sown in these plots at the optimum time for attack of root-rot. Three plots were covered by means of thatches, and high humidity and low temperature maintained in these plots by addition of water on the thatches at regular intervals throughout the day. The remaining three plots were not covered and served as control. Temperature and humidity records were taken twice a day at 8 A.M. and 5 P.M. both inside the covered and check plots. The mortality counts were taken at weekly intervals. The results of the experiment are given in Table III.

TABLE III

Effect of humidity and temperature on the incidence of root-rot disease

Week ending	Per cent mortality root-rot	Covered plots					Uncovered plots			
		Av. humidity	Av. air temperature (°F.)		Av.* soil temperature (°F.)	Per cent mortality root-rot	Av. humidity	Av. air temperature (°F.)		Av.* soil temperature (°F.)
			8 A.M.	5 P.M.				8 A.M.	5 P.M.	
7th June 1938	0	65·6	88·5	93·9	—	0	54·7	89·8	102·7	93·9
14th June 1938	0	79·5	81·7	88·7	—	0	69·9	84·3	83·3	86·7
21st June 1938	0	76·7	82·7	91·5	—	2·8	71·4	84·3	93·3	88·0
28th June 1938	0	75·2	83·1	92·6	84·8	2·5	67·1	86·6	94·3	92·6
5th July 1938	0	65·1	80·6	97·7	84·4	1·5	46·7	88·4	99·6	94·9
12th July 1938	0	65·2	85·6	98·0	86·2	5·3	52·3	91·0	102·9	96·0
19th July 1938	0	66·7	85·6	95·4	86·0	13·0	55·8	89·1	100·4	99·5
26th July 1938	0	81·5	80·6	88·0	85·9	8·3	75·9	85·7	91·9	96·6
2nd Aug. 1938	0	72·4	95·4	94·6	87·0	3·2	60·9	88·7	99·8	98·8
9th Aug. 1938	0	71·2	83·7	92·8	86·7	1·4	57·6	90·3	99·3	97·4
16th Aug. 1938	0	77·4	84·6	89·4	86·0	0·9	67·5	87·7	94·6	98·8
23rd Aug. 1938	0	77·1	83·4	90·8	85·6	0·9	63·6	86·6	97·1	95·5
30th Aug. 1938	0	87·8	82·8	87·7	84·3	0·0	77·6	84·6	92·1	93·8

* 30 cm. depth.

In the thatched plots no deaths occurred due to root-rot whereas in the uncovered control plots a large number of plants were killed as a result of root-rot attack from the third week of June to the third week of August. High humidity and low temperature in the covered plots appear to be unfavourable for the progress of the disease. It may, however, be mentioned that in the covered plots growth of plants was very poor.

C. Effect of mixed cropping on the incidence of the disease

Effect of mixed cropping was tested in a piece of land heavily infected with the disease. *Desi* cotton variety Mollisoni 39 was sown in rows three feet apart on 20th May, 1938, *i.e.*, the optimum time for the attack of the disease. Sorghum was sown on the same day in between the cotton rows. Root-rot mortality counts along with soil moisture determinations were made at weekly intervals both in the mixed crop and control in which sorghum had not been sown in between the cotton lines. The soil moisture estimations were made in one foot layers upto a depth of three feet. For estimation of moisture individual samples from different parts of the same plot were taken in order to get a fairly respresentative idea of the moisture content of the whole plot. Also air temperature and humidity was noted every day at 8 A.M. and 5 P.M. both inside the mixed crop and cotton.

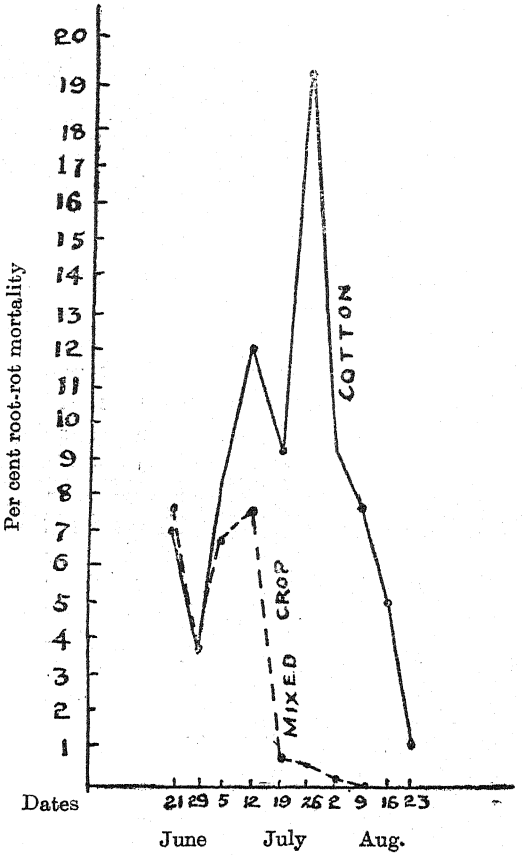

FIG. 1.

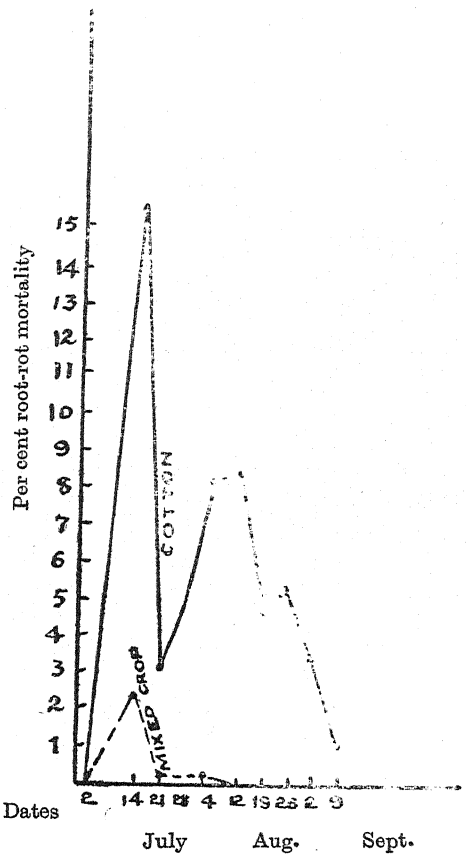

FIG. 2.

Effect of mixed cropping on root-rot mortality

The results of the experiment are set out in Table IV. The soil moisture figures show average percentage of soil moisture in three feet column of soil. Fig. 1 shows the effect of mixed cropping on the incidence of the disease.

The following are the chief points of interest :—

1. Mortality due to root-rot in the mixed crop is significantly lower than in the controls ; t being 2·98 [Fisher, 1925].

2. There is no regular difference in soil moisture content of the inter-cropped and control plots.

3. Humidity is higher in the mixed crop.

4. There is no appreciable difference in air temperature up to 28th June 1938 but during later part of the season air temperature is considerably lower in the mixed crop.

TABLE IV

Effect of mixed cropping on the incidence of root-rot disease

Week ending	Mixed crop (Cotton + sorghum.)					Cotton (Control)				
	Average per cent mortality root-rot	Average per cent soil moisture	Average per cent humidity	Average Air temperature (°F.)		Average per cent mortality root-rot	Average per cent soil moisture	Average per cent humidity	Average Air temperature (°F)	
				8 A.M.	5 P.M.				8 A.M.	5 P.M.
14th June 1938 .	7·5	—	86·8	85·4	94·1	6·8	—	66·8	85·4	94·1
21st June 1938 .		10·8	81·2	86·0	90·5		10·8	66·8	85·1	96·0
28th June 1938 .	3·4	8·8	67·9	87·4	96·3	3·4	9·7	56·7	87·8	97·8
5th July 1938 .	6·5	13·9	71·3	83·7	93·4	8·2	11·0	47·1	88·8	101·1
12th July 1938 .	7·3	8·4	60·0	89·8	99·7	11·5	10·8	45·2	93·5	105·7
19th July 1938 .	0·6	12·9	67·9	87·8	96·7	8·9	8·8	49·2	90·4	104·7
26th July 1938 .	0·5	10·0	85·2	85·1	90·1	19·1	12·0	76·9	86·4	91·4
2nd August 1938	0·2	8·0	66·7	87·9	98·0	9·0	9·9	53·9	90·4	104·0
9th August 1938 .	0·0	11·9	62·4	87·7	97·1	7·4	12·9	47·1	91·1	105·1
16th August 1938	0·0	8·9	71·1	86·3	92·9	5·0	8·8	65·2	88·0	94·3
23rd August 1938.	0·0	—	76·6	84·3	94·6	1·0	—	60·4	86·9	99·9

Sorghum plants up to 28th June 1938 were very small in size and did not serve as a cover to cotton plants, but as the season advanced there was a marked decline in the number of plants killed due to root-rot in the plots in which cotton was sown in mixture with sorghum. The sorghum plants had then grown tall enough to offer shelter to the cotton plants and were instrumental in lowering air temperature and raising humidity within the crop. It is, however, still a matter for further investigation whether sorghum can also serve as a barrier to the spread of the disease [Jordan *et al.*, 1934].

In another similar experiment sorghum was sown fourteen days before cotton so that sorghum plants could offer protection to cotton plants from the very beginning. Three repeats of both mixed and check plots were kept. Side rows of each plot were left as non-experimental rows. The data of this experiment are given in Table V. Fig. 2 shows the relation of mixed cropping to mortality due to root-rot.

TABLE V

Incidence of the disease in relation to mixed cropping

Week ending	Average per cent root-rot mortality	
	Mixed crop (Cotton + Sorghum)	Cotton (Check)
2nd July 1938 . . .	0·0	0·3
14th July 1938* . . .	2·3	15·2
21st July 1938 . . .	0·2	3·0
28th July 1938 . . .	0·0	5·0
4th August 1938 . . .	0·1	8·2
12th August 1938 . . .	0·0	8·3
19th August 1938 . . .	0·0	4·8
26th August 1938 . . .	0·0	5·2
2nd September 1938 . .	0.0	3·2
9th September 1938 . .	0·0	0·9
	$t = 3·31$	

*12 days mortality

The results show that root-rot mortality in the mixed crop is lower from the very beginning of the season and that the results are statistically significant.

D. Effect of soil composition

Root-rot disease of cotton invariably appears in patches. A few green healthy looking plants may be seen scattered all over the infected area, although they are usually plants which have been partially attacked by the disease, but have escaped death by putting out lateral roots in the uppermost layers of soil during wet weather or irrigation.

As healthy and diseased patches appear close to each other, examination of the soil was undertaken. It was considered possible that the information secured might help in finding why the disease causes great damage in one part of the field and does not do so in the adjoining portion.

Soil samples from healthy and disease bearing portions lying side by side in a plot were taken in one foot layers up to a depth of five feet. Samples were taken at Lyallpur, Khanewal and Sargodha. From each of the plots three individual samples were taken up to a depth of five feet and analysed separately, except in the case of Sargodha samples, where representative samples were obtained by thoroughly mixing up the corresponding samples from three different bores made in each healthy and diseased part. The analyses were carried out by the International method [1936].

(*i*) *Mechanical analysis*—Altogether one hundred and thirty samples from Sargodha, Lyallpur and Khanewal were analysed. The results of this analysis in Table VI do not show any regular differences in the texture of healthy and diseased soils.

(*ii*) *Estimation of nitrogen and organic matter.*—Nitrogen was estimated by the A. O. A. C. method and organic matter by Robinson and Mclean's oxidation method [1929]. C : N ratios were calculated from these. Results of these estimations which are given in Table VII do not show regular differences in the nitrogen and organic matter content and C : N ratio of healthy and diseased soil samples.

(*iii*) *Analysis of water extract.*—In this connection forty-eight samples were analysed and estimations were made for the total solids, carbonates, bicarbonates, sulphates, chlorides and calcium. No appreciable differences in any of the constituents estimated were however observed in healthy and diseased soil samples. The results of typical analyses are set out in Table VIII.

TABLE VI

Mechanical analysis of healthy and diseased soil samples

Depth	Sample*	Sargodha. Per cent						Lyallpur† Per cent						Khanewal† Per cent					
		Carbonates	Gravel	Sand	Fine sand	Silt	Clay	Carbonates	Gravel	Sand	Fine sand	Silt	Clay	Carbonates	Gravel	Sand	Fine sand	Silt	Clay
1st foot	H	1·85	0·14	7·70	62·89	16·70	12·68	0·76	—	3·99	69·66	16·86	9·58	5·36	—	2·92	68·30	18·50	10·26
	D	2·70	0·14	5·41	64·13	17·56	12·90	1·04	—	3·65	69·20	12·54	10·15	5·13	—	2·78	70·10	19·73	7·33
2nd foot	H	4·05	0·28	7·68	65·00	14·82	12·50	1·43	—	4·00	73·69	13·03	9·92	4·66	—	2·85	70·06	17·30	10·13
	D	6·15	—	5·61	68·69	14·00	11·60	1·95	—	3·35	72·09	13·71	11·93	4·53	0·12	2·59	73·20	19·10	8·53
3rd foot	H	5·47	0·28	7·78	66·62	14·00	11·60	2·40	—	2·43	72·96	15·02	9·57	4·66	—	3·86	70·50	15·66	9·66
	D	7·87	0·14	4·46	72·58	11·54	10·40	2·83	0·01	3·15	71·60	14·68	10·50	5·26	—	3·56	78·20	9·83	8·33
4th foot	H	6·86	1·68	6·37	68·15	14·09	11·40	2·61	—	2·30	70·57	16·54	10·20	3·86	—	4·72	73·10	13·55	8·65
	D	10·01	0·70	3·50	76·15	12·05	8·30	2·88	—	2·46	71·67	16·19	10·04	6·16	—	4·14	76·60	12·60	6·60
5th foot	H	9·77	14·70	6·50	67·25	15·45	10·80	2·95	0·38	2·37	67·70	20·42	9·46	4·96	2·40	3·47	67·90	19·63	8·93
	D	10·90	4·90	3·99	74·31	12·70	9·00	4·32	3·09	2·22	71·55	16·56	9·37	6·26	—	3·21	78·43	12·20	6·23

*H = Healthy, D = Diseased

†Mean of three samples

TABLE VII

Distribution of carbon and nitrogen in healthy and diseased soils

Depth	Sample*	Sargodha			Lyallpur†			Khanewal†		
		Per cent nitrogen	Per cent organic matter	C/N	Per cent itrogen	Per cent organic matter	C/N	Per cent nitrogen	Per cent organic matter	C/N
1st foot . . .	H	0·024	0·31	7·50	0·038	0·36	5·48	0·040	0·24	3·44
	D	0·028	0·37	7·80	0·036	0·33	5·50	0·031	0·23	4·47
2nd foot . . .	H	0·035	0·24	3·90	0·010	0·23	13·60	0·024	0·19	6·16
	D	0·024	0·21	5·00	0·019	0·25	11·30	0·028	0·19	3·89
3rd foot . . .	H	0·035	0·25	4·11	0·032	0·20	4·79	0·027	0·26	5·62
	D	0·024	0·23	5·54	0·013	0·24	15·10	0·031	0·16	3·07
4th foot . . .	H	0·028	0·23	4·86	0·016	0·21	7·70	0·027	—	—
	D	0·017	0·18	6·35	0·016	0·20	7·60	0·026	—	—
5th foot . . .	H	0·025	0·22	5·08	0·013	0·23	11·06	0·024	—	—
	D	0·024	0·20	4·79	0·021	0·21	8·60	0·023	—	—

*H = Healthy, D = Diseased

†Mean of three samples

TABLE VIII

Analysis of water extract of healthy and diseased soil samples

Sample		Total solids		Carbonates		Bicarbonate		Sulphates		Chlorides		Calcium	
Depth	No.	H	D	H	D	H	D	H	D	H	D	H	D
1st foot	1	0·056	0·096	*Nil*	*Nil*	0·084	0·100	Traces	*Nil*	0·049	0·084	—	—
	2	0·060	0·079	,,	,,	0·084	0·088	*Nil*	,,	0·052	0·049	—	—
	3	0·048	0·024	,,	,,	0·082	0·092	,,	,,	0·052	0·043	—	—
	4	0·070	0·048	,,	,,	0·092	0·092	,,	,,	0·055	0·032	—	—
2nd foot	1	0·080	0·080	,,	,,	0·065	0·061	—	—	0·095	0·031	0·019	0·022
	2	0·060	0·050	,,	,,	0·068	0·068	—	—	0·038	0·028	0·020	0·019
	3	0·158	0·010	,,	,,	0·076	0·068	—	—	0·039	0·032	0·019	0·017
	4	0·096	0·117	,,	,,	0·070	0·072	—	—	0·028	0·034	0·015	0·018
3rd foot	1	—	0·047	,,	,,	0·070	0·056	0·172	0·020	0·171	0·031	0·036	0·030
	2	0·044	0·054	,,	,,	0·070	0·073	0·012	0·012	0·047	0·034	0·019	0·017
	3	0·048	0·046	,,	,,	0·062	0·063	0·020	0·011	0·025	0·031	0·016	0·017
	4	0·048	0·210	,,	,,	0·079	0·063	0·020	—	0·031	0·041	0·022	0·084
4th foot	1	—	—	,,	,,	0·070	0·055	0·018	0·052	0·060	0·054	0·015	0·018
	2	—	—	,,	,,	0·060	0·068	0·010	0·038	0·050	0·043	0·014	0·015
	3	—	—	,,	,,	0·063	0·063	0·035	0·018	0·033	0·038	0·014	0·014
	4	—	—	,,	,,	0·061	0·045	0·024	—	0·035	0·032	0·014	0·062

(*iv*) *Chemical analysis.*—This included the determination of iron, aluminium, phosphorus, sodium, potassium, calcium and magnesium. Altogether twenty-four samples of diseased and healthy soil were analysed. The analysis did not show any appreciable difference in iron, aluminium, phosphorus, sodium and potassium contents of the healthy and diseased soil samples. Diseased soils, however, contain a higher amount of acid soluble calcium (t=3 and 5) and have a higher Ca : Mg ratio (t=3·5 and 3·2) than the healthy soils. The results of Ca : Mg estimations are given in Table IX.

TABLE IX

Calcium and magnesium contents of healthy and diseased soils (mg. per 100 grms.

	Sample	Healthy			Diseased		
		Ca	Mg	Ca/Mg	Ca	Mg	Ca/Mg
I	1st foot	1050	579	1·81	1322	579	2·28
	2nd foot	1287	1001	1·28	1858	1098	1·69
	3rd foot	1651	1647	1·0	3502	1357	2·58
	4th foot	1851	1454	1·27	4703	1387	3·39
	5th foot	1958	1514	1·29	4253	1261	3·37
II	1st foot	929	645	1·44	1858	585	3·17
	2nd foot	1029	989	1·04	2213	1158	1·91
	3rd foot	1701	1007	1·69	3102	1339	2·32
	4th foot	2251	1128	1·99	5003	1447	3·46
	5th foot	2251	1128	1·99	3902	1062	3·67

Although the roots of diseased plants contain comparatively higher amount of calcium, the percentage of calcium in diseased plants as a whole is lower than in the healthy plants [Vasudeva *et al.*, 1939]. The diseased plant soils, however, contain higher amount of calcium showing thereby that in the diseased plants calcium is not being transported to the upper parts. The picture would have been more complete had it been possible to work out a balance of calcium in the plant, the roots and the soil on which the plant has grown, but this is not possible with plants growing in the field since the soil concerned with the development of any individual plant cannot be considered as an isolated block. Further work in this direction is, however, necessary.

E. Effect of soil reaction

*p*H values of soil samples obtained both from healthy and affected fields were determined electrometrically and were found to vary from 5·4 to 8·6, the range over which the causal fungi, *R. solani* and *R. bataticola*, show good growth. No appreciable differences in *p*H values of healthy and diseased soil samples have however been observed. All the same an attempt was made to test the effect of changing soil reaction in an infected plot on the incidence of the disease. The reaction was changed by the addition of sulphur and lime to the soil and the different treatments were randomised. Six replications of each treatment were made. Lime and sulphur were first applied in 1936 but an additional dose had to be given in 1937 to set up a range of soil reactions varying from *p*H 5 to 8·5.

The data of this experiment showing the variations in soil reaction as a result of the addition of lime and sulphur and the average per cent mortality of cotton plants due to root-rot for three successive years are recorded in Table X. During 1937 germination of cotton seed was very poor in plots treated with sulphur which probably exerted a toxic effect on the seed.

TABLE X

Incidence of the disease in relation to soil reaction

No.	1936				1937			1938		
	Treatment	Dose per acre (lbs.)	*p*H	Average per cent root-rot mortality	Additional dose (lbs.)	*p*H	Average per cent root-rot mortality	Additional dose (lbs.)	*p*H	Average per cent root-rot mortality
1	Sulphur . .	1000	8·3	47·7	3000	7·4	31·4	—	—	56·7
2	Sulphur . .	3000	8·0	40·5	3000	5·9	24·9	—	—	55·7
3	Sulphur . .	4000	7·6	52·6	4000	5·1	30·5	—	—	47·0
4	Lime . . .	1000	8·7	43·4	3000	8·3	35·0	—	—	54·9
5	Lime . . .	2000	8·8	24·3	4000	8·2	31·2	—	—	51·4
6	Untreated Control .	—	8·6	38·1	—	8·2	30·0	—	—	59·1

The results given above indicate that the hydrogen ion concentration of the soil has no effect on the incidence of the disease.

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The junior author made some of the field observations under the guidance of the senior author.

IV. Summary

1. The bearing of temperature and humidity on the incidence of root-rot disease of cotton has been shown.

2. Preliminary observations indicate that virulent attack of the disease may be evaded by sowing cotton in mixture with sorghum.

3. There is no difference in the texture and chemical composition of healthy and diseased soils except that the diseased soils contain a higher amount of acid soluble calcium and have a higher Ca : Mg ratio.

4. There is no correlation between the hydrogen ion concentration of the soil and the incidence of the disease.

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THE AMARANTUS BORER, *LIXUS TRUNCATULUS* (F.) AND ITS PARASITES

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(With Plates XXVII and XXVIII)

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I. Introduction

The amarantus borer, as its name implies, bores into stems and branches of various species of cultivated and wild amarantus. Lefroy [1909] recognized, about thirty years ago, that *Lixus truncatulus* breeds freely in plants of amarantus, and Fletcher* [1914] considered it as a minor pest capable of doing considerable damage to cultivated amarantus ; yet very little was known of its life-history and control, while the method of oviposition and the nature and form of egg were altogether unknown.

Plants of the natural order Amarantaceae do not constitute staple crops but several species are cultivated as vegetable, ornamental or medicinal plants in many parts of India. Chief of these are :—

1. *Amarantus gangeticus* Linn.—Cultivated throughout India under the name of *lal sag*. Several varieties form an important vegetable of the country.

2. *Amarantus caudatus* Linn.—Cultivated in the south-western parts of India, also at Pusa. Leaves used as vegetable and the seed, called *ram dana*, employed for medicinal purposes.

Besides these useful species, there are several others such as *Amarantus viridis* Linn. and *Amarantus spinosus* Linn. which grow wild throughout India.

In October 1935, *Amarantus caudatus* growing in the kitchen garden at the Imperial Agricultural Research Institute, Pusa, was found severely attacked by the borer. This gave the writer an opportunity of studying the bionomics and potentialities of this pest. A number of parasites not so far known from this host have been bred from the immature stages of the borer. Some of these seem to be of considerable importance. The habits, status, life-history and morphology of the important ones have been worked out. A preliminary account of this work was communicated to the 24th Session of the Indian Science Congress and a brief account also appeared in the Annual Report of the Imperial Entomologist [Pruthi, 1936].

II. Biology and morphology of *Lixus truncatulus*

Distribution

The borer is widely distributed, occurring almost throughout the plains of India, from the Punjab in the west to Burma in the east and Bihar in the north to Madras in the south. Details of its distribution and food plants of the specimens contained in the Pusa, Coimbatore, Dehra Dun and Calcutta collections are given in Table I. It is clear that the insect occurs all the year round and is more abundant in South India than elsewhere.

*Fletcher records this species under the name *L. brachyrhinus* Bohem., which is now considered to be a synonym of *L. truncatulus* (Fabr.)

TABLE I

Distribution and food plants of Lixus truncatulus

Month	Locality and province	No. of individuals	Food plants and remarks
January	Dehra Dun (U. P.)	Several	*Amarantus spinosus**
	Nagpur (C. P.)	1	....
	Saidapet (Madras)	Several	*Amarantus* sp.*
February	Bombay	2	Wild grass
	Pusa (Bihar)	1	Oat leaf
	Coimbatore (Madras)	..	....
March	Dehra Dun (U. P.)	Several	*Amarantus spinosus**
	Bombay	1	....
	Daltonganj (Bengal)	1	*Zizyphus jujuba*
	Coimbatore (Madras)	..	....
April	Dehra Dun (U. P.)	Several	*Amarantus spinosus**
	Surat (Bombay)	1	....
	Lahore (Punjab)	1	*Amarantus* sp.
	Pusa (Bihar)	5	*Amarantus spinosus**
	Ganjam (S. India)	Several	At light.
	South Arcot (S. India)	..	*Amarantus* sp.
May	Purneah (Bengal)	..	....
	Pusa (Bihar)	1	Indigo
	Pusa (Bihar)	1	*Amarantus* sp.
	Lahore (Punjab)	2	*Amarantus* sp.
	Coimbatore (Madras)	..	....
June	Pusa (Bihar)	1	....
	Coimbatore (Madras)	1	*Babul*
	Madras	Several	*Amarantus gangeticus*
July	Siliguri (Bengal)	..	....
	Calcutta (Bengal)	..	....
	Darjeeling (Bengal)	..	....
	Ballygunj (Bengal)	..	At light
	Mandalay (Burma)	2	*Amarantus* sp.
	Kalka (Punjab)	1	....
	Pusa (Bihar)	3	....
	Coimbatore (Madras)	..	*Babul*
August	Purneah (Bengal)	..	....
	Pusa (Bihar)	2	*Amarantus gangeticus**
	Upper Burma	3	....
	Trichinopoly (Madras)	1	*Amarantus* sp.

* In these cases the immature stages were found actually in the food plants mentioned.

TABLE I—*concld.*

Month	Locality and province	No. of individuals	Food plants and remarks
September .	Dehra Dun (U. P.) . .	Several	*Amatantus gangeticus**
	Monghyr (Bihar) . .	..	....
	Pusa (Bihar) . . .	2	*Amarantus gangeticus* *
	Saidapet and Vizag. (Madras).	7	*Amarantus* sp.
October . .	Dehra Dun (U. P.) . .	..	*Amarantus gangeticus**
	Chingliput (Bombay) .	1	*Amarantus* sp.
	Cawnpore (U. P.) . .	2	Niger
	Surat (Bombay) . .	1	....
	Nagpur (C. P.) . .	1	Cotton
	Bilaspur „ . . .	1	....
	Pusa (Bihar) . . .	4	*Amarantus* sp.*
	Madhopur (Bengal) . .	..	....
	Attur, Annampali, Godavary (S. India).	..	*Amarantus* sp.*
November .	Dehra Dun (U. P.) . .	Several	*Amarantus spinosus**
	Pusa (Bihar) . . .	2	*Cucurbitaceae*
		3	*Amarantus* sp.
	Gurdaspur (Punjab) . .	1	....
	Cuttack (Bengal) . .	1	....
December .	Gorakhpur (U. P.) . .	1	....
	Trichinopoly (Madras) .	1	....
	Godavary (Madras) . .	..	....

Nature of damage and food plants

After hatching under the epidermis of a twig, the young grub bores towards its pith. On reaching the pithy portion in the centre it bores along the length of the branch, the passage of least resistance. It is, however, interesting to find that the grub almost invariably bores towards the proximal end which is the thicker end of the branch. Arriving at the junction it burrows into the next thicker branch; in this way increase in the size of the grub automatically synchronises with increase in the thickness of the branch. When full grown, the grub prepares a wide chamber for pupation. This is marked by a characteristic swelling of the stem externally. In severe cases of attack one may find a large number of such swellings marking the position of full-grown grubs and pupae. From a single large plant of *Amarantus caudatus* 155 individuals of this species in different stages of development were collected. Fletcher recorded only ten to twelve grubs infesting a single host plant. As a result of such a heavy infestation the plant growth becomes

* In these cases the immature stages were found actually in the food plants mentioned.

stunted, and there are but few tender shoots and new leaves left on the plant which are the chief portion used as vegetable. All the old branches and main stem present a twisted and swollen appearance and finally there is very limited flowering and setting of seed.

Though a pest of amarantus, the weevil has also been reported on a variety of other plants, *e.g.*, niger, indigo *Zizyphus jujuba*, *Lagenaria vulgaris*, *Solanum melongena*, etc. It is interesting to note that with the exception of amarantus, the larval stages are not known from any other host plant.

In order to determine the actual food plants of this weevil, fivepairs of adults, emerged between 6th and 9th December, 1935, were caged on different species of *Amarantus* and other allied plants. Before introducing the weevils, the parts of the plant enclosed were examined carefully for eggs and grubs. Twigs showing oviposition or damage, such as nibbled leaves, etc., were rejected. After every twenty-four hours the weevils were transferred to a fresh twig, the previous one being examined for eggs as well as damage to leaves. The experiments repeated six to eleven times with each food plant show that all the four species of *Amarantus*, viz., *gangeticus*, *caudatus*, *viridis* and *spinosus*, are attacked by the weevil, their leaves and tender branches are eaten by the adults, eggs are laid on them and the grubs feed inside their stem. *Amarantus spinosus* is the least susceptible; eggs were laid only in three cases out of eight and the leaves were only slightly nibbled. This is supported by field observations, which show that when other species of *Amarantus* are available, plants of *Amarantus spinosus* in the same locality are quite healthy and free from attack. They are only attacked in case the other varieties are absent or scarce. In the case of *Chenopodium album* the leaves were reluctantly nibbled after some starvation, and eggs were laid in the stem after boring several empty holes. The young grubs in this case were found to develop inside the stem to some extent but could not be reared to the adult stage. It seems that the weevil infests *Chenopodium album* and *Amarantus spinosus* under conditions of scarcity of suitable species of *Amarantus*. The leaves of *Zizyphus jujuba* were slightly nibbled by the adult weevils but neither eggs were laid on it nor were the grubs found inside the stem. On *Lagenari vulgaris* and *Solanum melongena* the weevils could neither feed nor oviposit.

Emergence, copulation and pre-oviposition period

This weevil is a rather slow-developing insect with overlapping broods in nature; the adults continue emerging from the infested plants without any definite interruption.

In order to get an idea of the rate of their emergence, five full-grown plants of *Amarantus caudatus* were caged in two separate wire-gauze cages and the adult weevils emerging therefrom were collected and recorded. It was noticed that the weekly number of adults emerging from the end of December to middle of January was somewhat higher than that emerging during the rest of the period, but there was no definite break in emergence during the experimental period.

The weevils are almost sexually mature after emergence, therefore the pre-copulation and pre-oviposition periods are short. Even in December, the weevils emerged on the 9th began to copulate on the 13th and started egg-laying on the 14th. The copulation lasts for about seven to eight hours and egg-laying may commence immediately thereafter.

Oviposition.—As stated already, the eggs and the method of oviposition were so far unknown. Fletcher [1917] surmised that the eggs are probably laid inside the stem under the axil of a leaf on one of the lateral branches. It has now been definitely observed that the female bores a hole, 1-1·5 mm. deep, in the tender branches of the plant or in the petiole or even midrib of leaves, and deposits a single egg therein (Plate XXVII, fig. 2). The mouth of the hole is closed with a sticky secretion spread into an oval flap. When fresh, this flap is dull green in colour and, therefore, it is difficult to make out the seat of oviposition without a careful examination. In a few days, the flap becomes black (Plate XXVII, fig. 1), but by this time the egg has already hatched and the grub bored into the stem. Older branches of plants, which are usually hard and somewhat dry, are avoided for oviposition.

During favourable season, *i.e.*, from April to September, the female lays two to three eggs per day (maximum six eggs) while during winter egg-laying may stop altogether. The maximum number laid by one female was found to be 174 spread over a life of about five months. The oviposition record of a typical female showed that during the first month following emergence, *viz.*, 9th December to 8th January, it laid twenty eggs. During the next month on account of severe cold, oviposition stopped altogether. It then laid sixty-four eggs during March and forty-nine eggs during April and died on 4th May after an oviposition period of about five months.

Egg.—The egg is oblong, broadly rounded at the two ends and measures on an average 1·2 mm. in length and 0·7 mm. in breadth (Plate XXVII, fig. 3). It has a uniformly pale yellow colouration and a smooth glistening surface. As it develops, it undergoes little change in shape or colour except that the length increases slighly at the expense of the breadth. Forty-eight hours after oviposition at 20°C., the dark reddish brown mandibles of the embryo become visible. They grow more and more prominent as hatching approaches and an hour before actual hatching they show a gnawing movement. As the eggs are laid in contact with cell sap of the plant, they require for their development not only a saturated atmosphere, but perhaps direct contact with free water. Thus the eggs removed from the plant and kept in a partially saturated atmosphere did not hatch at all. At room temperature, during March and November, the incubation of eggs takes ten to twelve days. Freshly laid eggs kept at constant temperatures of 27°C. and 20°C. hatched in about four days and ten days respectively.

Grub.—The freshly hatched grub (Plate XXVII, fig. 4) is smaller than the egg. It measures about 1·0 mm. long and 0·5 mm. broad. The head capsule is large, as broad as long, and constitutes about one third of the body length. It is light brown with several darker transverse streaks. The mandibles are

PLATE XXVII

FIG. 1. Dark spot showing the seat of oviposition by *Lixus truncatulus*
FIG. 2. Cross section of twig showing an egg of *Lixus truncatulus* inside the hole (×4)
FIG. 3. Egg of *Lixus truncatulus* taken out of the hole (×10)
FIG. 4. Grub of *Lixus truncatulus*, freshly hatched (×20)
FIG. 5. Pupa of *Lixus truncatulus*, (×6)
FIG. 6. Adult of *Lixus truncatulus*, (×8)
FIG. 7. Larva of *Lestodiplosis* sp. on weevil egg (×6)
FIG. 8. Adult of *Lestodiplosis* sp. (×20)
FIG. 9. Covering of hole cut open to show (a) Egg of *Paracudeva tolymoides*, and (b) the weevil egg (×4)
FIG. 10. Adult of *Dinarmus acutus* ♀ (×16)
FIG. 11. Adult of *Dinarmus acutus* ♂ (×16)

PLATE XXVII

FIG. 1. Dark spot showing the seat of oviposition by *Lixus truncatulus*

FIG. 2. Cross section of twig showing an egg of *Lixus truncatulus* inside the hole (×4)

FIG. 3. Egg of *Lixus truncatulus* taken out of the hole (×10)

FIG. 4. Grub of *Lixus truncatulus*, freshly hatched (×20)

FIG. 5. Pupa of *Lixus truncatulus*, (×6)

FIG. 6. Adult of *Lixus truncatulus*, (×8)

FIG. 7. Larva of *Lestodiplosis* sp. on weevil egg (×6)

FIG. 8. Adult of *Lestodiplosis* sp. (×20)

FIG. 9. Covering of hole cut open to show (*a*) Egg of *Pareuderus torymoides*, and (*b*) the weevil egg (×4)

FIG. 10. Adult of *Dinarmus sauteri* ♀ (×16)

FIG. 11. Adult of *Dinarmus sauteri* ♂ (×16)

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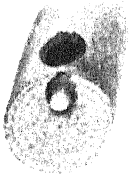

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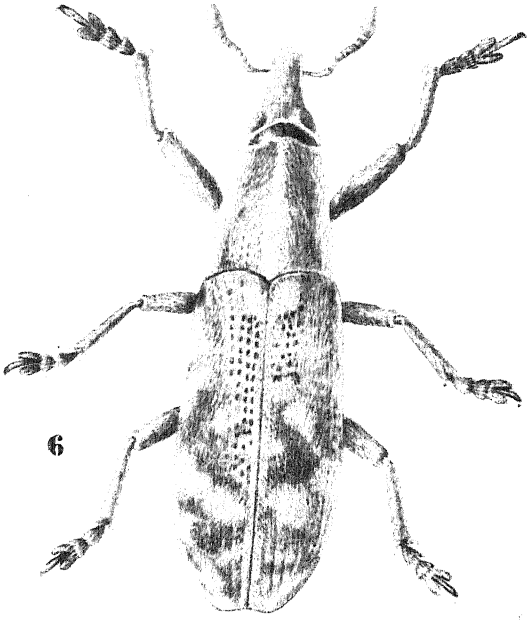

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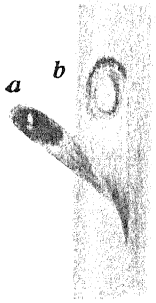

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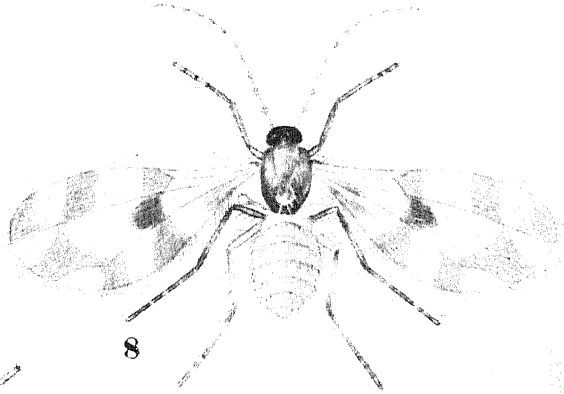

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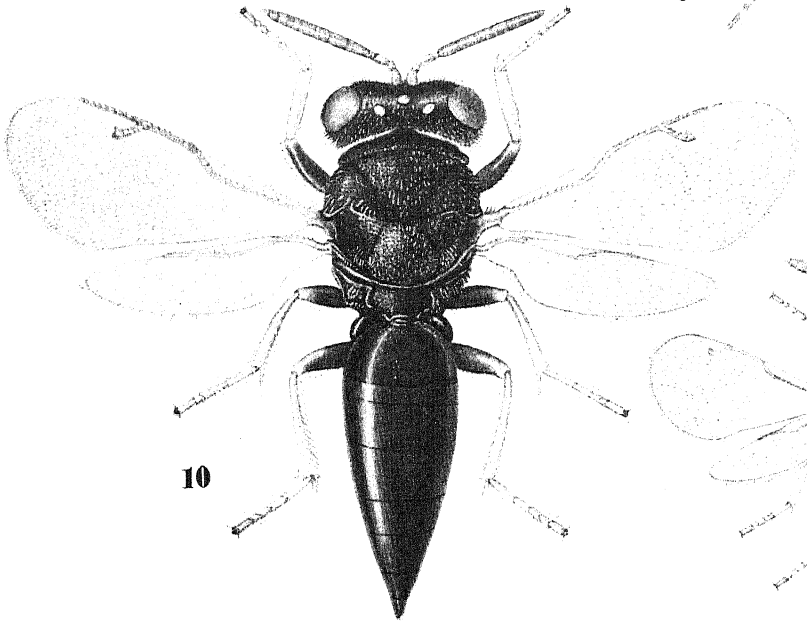

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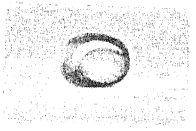

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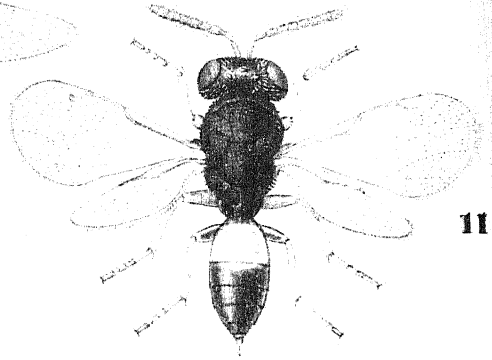

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strong and dark brown in colour. The thorax and abdomen are uniformly light yellow. All the segments are nearly equal in breadth and size, excepting the last two abdominal segments which successively decrease in size. The terminal part of abdomen is thus broadly rounded dorsally.

The full-grown grub measures 14-15 mm. in length and 1·7 to 1·8 mm. in breadth. The description of full-grown grub as given by Gardner [1934] is reproduced below :—

" Body curved, slightly wider at thorax, posteriorly broadly rounded. Skin densely micro-asperate. Length about 15 mm. Head capsule reddish-testaceous with broad pale streaks, width about 1·75 mm. Frons wider than long, without dark median line. Ocelli one near antenna and a small posterior black spot on each side. Antenna with apical segment stoutly conical. Epipharynx with a row of six or seven long antero-lateral setae on each side, the stout rods contiguous posteriorly. Apical segments of palps small but distinctly longer than wide, labial palps separated by about twice width of basal segment. Typical abdominal terga with four distinct transverse folds, the fourth not protuberant. Spiracles nearly circular, with paired air-tubes projecting well beyond peritreme, posterior pair widely separated, subequal to preceding pair."

The grub stage of the weevil being a period of rather slow development, all attempts to complete this stage inside the constant-temperature incubators failed, as it was not possible to keep the growing plants in a healthy condition for sufficiently long period in the darkness of the incubator. On the other hand, the process of frequent transferring of the grub from old to fresh twig resulted, as in the case of many other borers, into extremely slackened development, often followed by death. Under conditions of room temperature, however, the grub stage, though very variable, was completed in several cases and the data are given in Table II. It will be noticed that grubs hatched about the end of March, pupated after forty to fifty-five days, while those which hatched in the end of October and beginning of November passed the entire winter in the larval stage.

TABLE II

Larval period of Lixus truncatulus *at room temperature*

Ref. No.	Date of hatching	Date of pupation	Pupal period (days)
17	30th Oct. 1935 .	Died on 15th Mar. 1936 without pupating	..
18	9th Nov. 1935 .	Died on 9th Feb. 1936 without pupating	..
19	26th Mar. 1936 .	20th May 1936	55
21	28th Mar. 1936 .	15th May 1936	48
24	5th Apl. 1936 .	24th May 1936	53
27	9th Apl. 1936 .	19th May 1936	40
27	9th Apl. 1936 .	24th May 1936	45
29	12th Apl. 1936 .	2nd June 1936	51

It is remarkable that the grubs can stand considerable starvation, particularly during the cold weather. A lot of fifteen well-developed grubs collected

from amarantus stem on 4th December 1935 was kept without food in the laboratory. Excepting two grubs which died during the first week, the rest died between 22nd February and 3rd March 1936. Thus the maximum period of starvation was as long as eighty-nine days.

Pupa.—When full grown, the grub prepares a wide chamber for pupation which causes a characteristic swelling of the stem or gall. The pupa (Plate XXVII, fig. 5) measures 8·7 to 9·0 mm. in length and about 2·6 mm. in maximum width. The eyes are dark from the very beginning, the rest of the body is pale yellow with dark brown lines at the inter-segmental areas of abdomen and antennae and along veins of the wing pads. The wing pads lie between the second and third pair of folded legs and extend a little beyond the tibio-femoral fold of third pair of legs. The abdomen is straight with prominent circular spiracles on the lateral aspect of segments.

Ordinarily the pupa lies motionless, but when disturbed, it moves its abdomen actively and can wriggle about a little. The duration of the pupal period as shown in Table III is nine to ten days at 27°C. as well as at room temperature during April, and twenty to twenty-four days at 20°C.

TABLE III

Pupal period of Lixus truncatulus *at different temperatures*

Ref. No.	Temperature	Date of pupation	Date of emergence of adults	Pupal period (days)	No. of weevils emerged
41	27°C.	9th Dec. 1935	18th December 1935	9	3
			19th December 1935	10	5
42	27°C.	17th Jan. 1936	26th January 1936	9	7
			27th January 1936	10	6
			28th January 1936	11	1
128	20°C.	1st Feb. 1936	21st February 1936.	20	2
			22nd February 1936	21	2
			24th February 1936	23	4
129	20°C.	3rd Feb. 1936	24th February 1936	21	1
			25th February 1936	22	5
			26th February 1936	23	3
			27th February 1936	24	2
136	Room temperature	5th Apl. 1936	15th April 1936	10	5
			16th April 1936	11	2
137	Do.	9th Apl. 1936	18th April 1936	9	3
			19th April 1936	10	4
			20th April 1936	11	1

Adult weevil.—In general appearance the weevil (Plate XXVII, fig. 6) is dirty coloured with several dark patches on a densely pubescent body. It is rather large in size, measuring on the average 8·75 mm. long and 3 mm. broad. Though very sluggish and disinclined to fly or even run, it is quite alert in noticing an approaching hand. By a little but prompt movement it just turns round the stem away from the hand and gets concealed by falling on the ground and feigning death like many other Curculionidae. On falling down it lies ventral surface upwards and the legs stretched out. The ventral surface being uniformly dirty white matches exactly with the ground colour and thus seems to offer a successful protection against enemies.

When food is available the life of the adults is fairly long. A great majority of the weevils emerging in November and December lived up to the month of April and May next, thus obtaining a life of four to six months.

When kept without food the life of the weevils is considerably shortened. A number of freshly emerged weevils was starved at a constant temperature of 27°C. and two different saturation deficiencies. It was noticed that the average life of starved adults was 9·2 days (maximum 15 days) at a saturation deficiency of 9 mm. and 7·6 days (maximum 12 days) at a saturation deficiency of 0 mm. This shows that fully saturated atmosphere is detrimental to the longevity of adults, whereas it was essential for the proper development of eggs and grubs.

Number of broods.—From the foregoing account of the rate of development, it will be observed that under favourable conditions the total pre-imaginal period is roughly two months. This means that there are at least three broods from March to November. As already pointed out, owing to the rather irregular and variable larval development, the different broods in nature are not distinct. During winter all the stages of the weevil are found undergoing slow but some development during the day. Thus there is no true hibernation.

III. PARASITES AND PREDATORS OF *LIXUS TRUNCATULUS*

From the account of the life-history of *Lixus truncatulus* it will be observed that all its pre-imaginal stages are passed within the host plant; even the eggs are laid inside the plant tissue and covered over with a layer of sticky secretion. This would indicate that the weevil leads a well protected life from its enemies. Actually, however, it was found that it is prone to the attacks of a number of parasites, particularly in the egg stage. These are listed below :—

1. Egg parasites—
   (1) *Pareuderus torymoides* Ferriere
   (2) *Anastatus* sp. prox. *coleopterorum* Girault
   (3) *Anastatus* sp.
   (4) *Lestodiplosis* sp.
   (5) *Telenomus javensis* Dodd

2. Larval parasite—

(6) *Dinarmus sauteri* Masi

3. Pupal parasite—

(7) *Eurytoma curculionum* Mayr

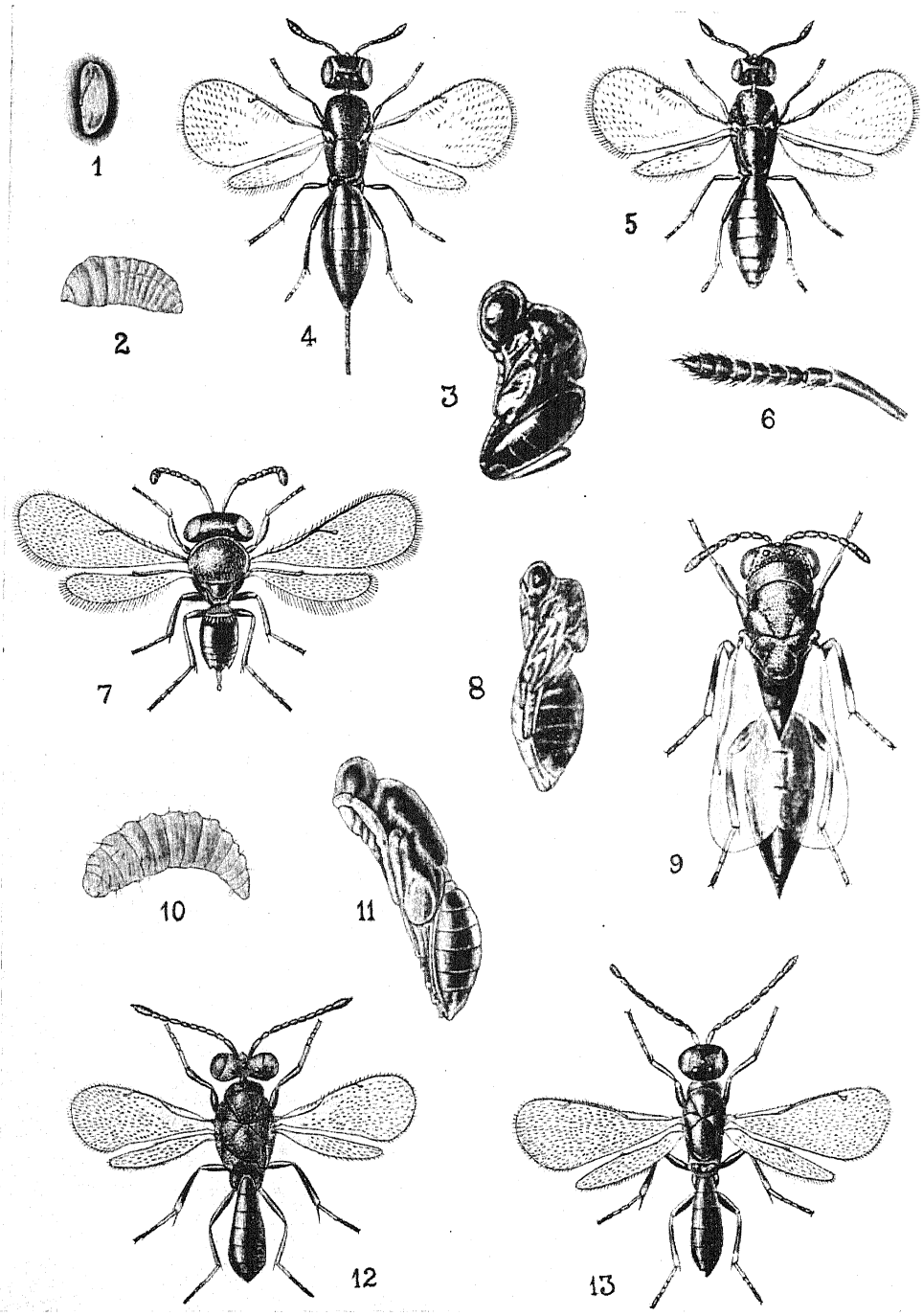
Recently Gardner (unpublished) has reared a species of *Calliephialtes* at Dehra Dun and Ayyar [1938] has bred some parasites of the genera *Tetrastichus*, *Dinarmus* (*?*) and *Spathius* from the immature stages of the weevil. Thus it is noteworthy that all the parasites dealt with in this paper have been bred from *Lixus truncatulus* for the first time, and were not known from Indian region so far. A detailed account of the life-history and morphology of the important ones is given in the following pages.

1. Pareuderus torymoides *Ferriere* (*Eulophidae* : *Chalcidoidea*)

Of the five egg parasites, listed above, *Pareuderus torymoides* Ferr. was found to be the commonest, and parasitised a larger proportion of eggs than those killed by all the other parasites put together. This is the first record of the species from India and perhaps the second in the world. It was only a few years ago [Ferriere, 1931] that the parasite was described from Java where it was found parasitising the eggs of *Alcides leeuwanii* infesting *Ceiba pentendra*. Beyond this first report of occurrence nothing is known of the life-history, status, etc., of the parasite. We have several species of *Alcides* injurious to vegetables in India and it is very likely that, as our knowledge advances, several additions will be made to the hosts of this apparently important parasite.

Systematic position and diagnostic characters.—This species is the genotype on which Ferriere [1931] erected the genus *Pareuderus* which is closely allied to *Euderus*. In order to facilitate the distinction of the species its important characters extracted from author's description are given below.—

" Head and thorax black with some bluish reflections or sometimes greenish on the thorax of males ; abdomen dark greenish or bluish violet on the sides. Antennae dark brown, pedicel with bluish shine, underside of scape yellow. Legs with bluish coxae and femora, the tibiae brown slightly bluish in the middle, the tip of tibiae and the tarsi white, except the last tarsal joint which is brown. Female (Plate XXVIII, fig. 4) : below the front ocellus a transverse furrow stretches almost from one eye to the other, separating the vertex from the frons. Face shagreened, slightly convex, the antennal groove not deep and without marginal carinae. Cheeks with length one third the length of an eye. Antennae inserted in the middle of the face, pedicel not broader than, and as long as, a third of the scape ; the four joints of the funicle subequal in length and as long as the pedicel ; club longer than the two preceding joints together, pointed at tip. Thorax entirely punctate, dull, the scutellum and the pleurae more finely punctate than the mesonotum, scutellum not higher than the mesonotum, but strongly convex on the sides and behind. Propodeon short, punctate, with a median carina and short furrows near the spiracles. Marginal vein longer than the submarginal, gradually and slightly narrowed toward the apex ; postmarginal vein thin, as long as one fourth of the marginal ; stigmal vein shorter than the postmarginal. Male (Plate XXVII, figs. 5 & 6): similar but the scutellum more convex at the sides and behind. Length : ♀ 1·5-1·6 mm. (without ovipositor) ; ♂ 1·2-1·4 mm.



FIGS. 1—6. *Pareuderus torymoides*— Fig. 1. Egg (×25), Fig. 2. Grub (×15), Fig. 3. Pupa (×25), Fig. 4. Adult, female (×25), Fig 5. Adult, male (×25), Fig 6. Antenna of male (×100)

FIG. 7. Adult ♂ of *Telenomus javensis* (×25)

FIGS. 8 & 9. *Eurytoma curculionum*—Fig. 8. Pupa (×8), Fig. 9. Adult (×12)

FIGS. 10—12. *Anastatus* sp.— ♂ Fig. 10. Grub (×15), Fig. 11. Pupa (×25), Fig. 12. Adult (×22)

FIG. 13. Adult of *Anastatus* sp. prox. *coleopterorum* ♂ (×25)

Life-history and description of various stages.

Oviposition.—The female parasite locates the egg cavity of the host, pierces the lid of the cavity with its ovipositor and deposits a single egg just above the host egg (Plate XXVII, fig. 9). It is interesting to note that the parasite is capable of discovering the host egg which is laid so securely inside the plant tissue.

The freshly laid egg (Plate XXVIII, fig. 1) is semi-transparent, becoming more opaque and whitish as development advances. It measures 0·36 mm. in length and 0·17 mm. in breadth. It is broadly rounded at the anterior end and somewhat narrowed posteriorly. At the broad end it carries a stalk, usually curved and about half as long as the egg. Due to its elastic shell the egg does not maintain symmetrical shape but under pressure one side may become more convex than the other.

At room temperature during December (17°-21°C.) egg hatches in about two days; at a constant temperature of 27°C. it hatches within twenty-four hours.

Grub.—Soon after hatching the grub goes underneath the host egg and begins to feed on the egg contents. Evidently the puncture made is so fine that egg contents do not ooze out immediately, and the egg retains its shape for a few days. Gradually the egg loses its turgidity, till it becomes empty and a well-developed parasite grub is seen under the host egg shell. One host egg is enough for the entire feeding stage of one parasite.

The well-developed grub (Plate XXVIII, fig. 2) measures about 1 mm. in length and 0·5 mm. in breadth. Body consists of a small head, three thoracic and ten abdominal segments. The first thoracic segment is the largest, the second and third are about half as broad as the first. The abdominal segments are much narrower than the thoracic segments so that the ten abdominal segments put together constitute only about half the entire length of the grub. The entire body of the grub is white with a faint bluish tinge. The sharp mandibles are, however, dark brownish.

The duration of the grub stage is rather short; it is five to six days at a constant temperature of 27°C. and about ten days at room temperature (17°-21°C.) during December.

Pupa.—The full-grown grub pupates within the cavity in which the host egg was laid. The pupa is stout, broad and very much curved (Plate XXVIII, fig. 3). The eyes are round and comparatively large and the antennae are rolled over the eyes reaching as far as the middle of abdomen. It measures about 1·25 mm. in length and 0·66 mm. in breadth.

The pupal period was determined from a large number of individuals, of which some typical cases are given below (Table IV) :—

TABLE IV

Pupal period of P. torymoides

Ref. No.	Date of pupation	Date of emergence	Pupal period (days)	Temperature (°C.)
43	13th December 1936 .	18th December 1936 .	5	27
44	14th December 1936 .	19th December 1936 .	5	27
54	20th December 1936 .	25th December 1936 .	5	27
61	21st December 1936 .	25-26th December 1936	5-6	27
70	25th December 1936 .	30th December 1936 .	5	27
74	25th December 1936 .	30-31st December 1936 .	5-6	27
90	4th January 1937 .	8th January 1937 .	5	27
		9th January 1937 . .	6	27
116	18th January 1937 .	24th January 1937 .	6	27
119	19th January 1937 .	24th January 1937 .	5	27
122	27th January 1937 .	4th February 1937 .	8	20

It will be noticed that the pupal duration is about five to six days at 27°C. and nearly eight days at 20°C.

Number of generations.—From the data given above we find that the pre-imaginal stages of the parasite are completed in less than a fortnight at 27°C. and in about three weeks during December (temperature 17°-21°C.). The host, on the other hand, is very slow developing; it takes over two months even under favourable conditions of temperature, *viz.*, 27°C. It has not been possible to study all the factors, but from the work carried out so far, it seems that the parasite has sufficient potential capacity to control the multiplication of the host. Under natural conditions during winter about 31 per cent of the weevil eggs were found parasitised.

2. Anastatus *sp. prox.* coleopterorum *Girault.*

3. Anastatus *sp.*

(*Encyrtidae* : *Chalcidoidea*)

The weevil egg is parasitised by two closely allied species of *Anastatus*, the life-history of which is somewhat similar to, but the incidence much lower than, that of *Pareuderus torymoides* F. Of these, *Anastatus* sp. prox. *coleopterorum* is comparatively more common than the other. Otherwise their life-histories are similar and are therefore dealt with together.

Oviposition.—Eggs are laid singly just under the lid of the cavity containing the host egg. The egg is translucent white, resembling in shape with, but slightly bigger than the egg of *P. torymoides*. It measures 0·44 mm. in length and 0·15 mm. in breadth. When about to hatch, the egg becomes slightly elongate.

Grub.—In about forty-eight hours the grub hatches out of the egg, goes under the host egg and begins to feed on it. After some time the host egg

loses its usual brilliance and turgidity and becomes dull and depressed. The parasite grub feeds so rapidly and voraciously for the first two to three days that by this time the host egg seems almost entirely consumed. Later, the feeding and development are slow, the larval period being completed in five to seven days at 27°C. (Table V). One egg is sufficient for the completion of the larval stage of the parasite. Superparasitism was noticed in one instance when a full-grown grub of *Anastatus* sp. was found along with a pupa of *Pareuderus torymoides* in a hole containing remnants of the host egg. The two parasites can probably develop together without detriment to each other and two individuals can complete development on a single egg of the host.

TABLE V

Larval and pupal periods of Anastatus *spp.*

Ref. No.	Temperature (°C.)	Date of hatching	Date of pupation	Larval period (days)	Date of emergence of adult	Pupal period (days)
64	27	22nd December 1935.	29th December 1935.	7	4th January 1936	6
81	27	31st December 1935	5th January 1936	5	11th January 1936	6
83	27	..	1st January 1936	..	8th January 1936	7
97	27	6th January 1936	12th January 1936	6	18th January 1936	6
99	27	..	7th January 1936	..	13th January 1936	6
100	27	9th January 1936	14th January 1936	5	20th January 1936	6
109	27	17th January 1936	22nd January 1936	5	29th January 1936	7
117	27	..	19th January 1936	..	26th January 1936	7
118	27	..	20th January 1936	..	27th January 1936	7
			Average .	5·6		6·4

The full-grown grub (Plate XXVIII, fig. 10) is elongate, measuring 1·5 mm. in length and 0·5 mm. in breadth. The head is large, glistening-white and turned downwards. The three thoracic segments bear a row of five to six long setae medially on either side of the body. The maximum thickness of, the body is maintained up to the first five abdominal segments which are darker than the rest of the body owing to the food contents inside. The posterior segments of the abdomen gradually taper to the narrow anal and are smoky white in colour.

The grub leaves feeding a few hours before pupation which takes place within the plant cavity.

Pupa.—The pupa (Plate XXVIII, fig. 11) differs very markedly from that of *P. torymoides*, being more slender and longer as well as more erect than the latter. It is 1·5 mm. long and 0·5 mm. broad in the region of the thorax. The eyes are comparatively small, the antennae not reaching as far as the distal

end of folded wings and the legs reaching up to the tip of the abdomen. The abdominal segments are quite distinct.

The pupal period lasts six to seven days at a temperature of 27°C. (Table V).

Adult.—It will be desirable to give here briefly the distinguishing features of the two species of *Anastatus*.

Anastatus sp. prox. *coleopterorum* Girault ♂ (Plate XXVIII, fig. 13): The adult is comparatively smaller, measuring 1·1-1·25 mm. long and about 0·25 mm. broad in the region of the thorax. Head and thorax are metallic green and the eyes are coppery red. Front femora are dark green while mid and hind femora are dark brown. The front tibiae and tarsi are brownish; also mid and hind tibiae, except at apex, are brown. Basal three segments of tarsi are light brown, while the terminal two segments are darker. Axillae almost meet mesad. Marginal vein in front wings is somewhat equal to the submarginal vein. The abdomen is dark brick-red. Lateral ocelli are separated from the eye border by a distance equal to that between the front and the lateral ones.

Anastatus sp. ♂ (Plate XXVIII, fig. 12): Length 1·25-1·50 mm., breadth about 0·5 mm. This species is readily separated from the above by its little larger size and uniform dark brown or black colour. Lateral ocelli are separated from eye border by a distance much less than that between the front and the lateral ones. Eyes are dark brown. The axillae are distinctly separate mesad. Legs except fore tibiae and tarsi and apices of mid and hind tibiae are concolourous with thorax. Except metatarsus, rest of the segments of mid and hind tarsi are dark. Thorax is black and relatively broader than in the other species. Marginal vein is somewhat shorter than the submarginal vein. Abdomen is dark brown.

Number of generations and incidence.—From Table V it will be observed that these parasites complete the pre-imaginal period, *i.e.*, from oviposition to emergence of adult, in less than two weeks at 27°C., the temperature obtaining during April-May at Pusa. In spite of the fact that they develop almost as fast as *P. torymoides* their incidence in nature remains very low throughout. This shows that other factors of environment are not suitable for their rapid multiplication.

4. Telenomus javensis *Dodd*

(*Scelionidae* : *Proctotrypoidea*)

A number of adults of the Scelionid parasite, *Telenomus javensis* Dodd (Plate XXVIII, fig. 7), emerged in cages containing *Amarantus caudatus* twigs infested with different stages of *Lixus truncatulus*. The exact status of the parasite and the stage of the host attacked are not known but previous records show that the whole group of Scelionidae comprises egg parasites without excep-

tion. It is, therefore, very likely that here too it attacks the egg stage of the weevil. The parasite is recorded from Java, where it was bred in 1914 from the eggs of a Tineid infesting sugarcane. Kieffer's [1926] revised description of the female of this species translated into English is given below :—

" Black. Head a little broader than the thorax. Eyes finely pubescent. Antennae 11-segmented, scape as long as the five following segments put together, length of second segment hardly twice its thickness, third segment much smaller but hardly thinner than the second, somewhat longer than thick, 4-6 segments almost similar, transverse. Club five segmented, its proximal segment short, proximal four segments transverse, the third being considerably thicker than the rest. Thorax just longer than broad, pubescent, and finely and irregularly sculptured. The fore wings reaching much beyond the tip of abdomen, hyaline, not very broad, marginal ciliation fairly long, surface hairs fine and thick, veins yellowish, the submarginal vein interrupted in the middle of the front margin, marginal vein half as long as the stigmal vein. Femur dark brown, tibia brownish yellow, tarsi yellow. Abdomen a little longer but not broader than the thorax, truncate apically, first tergite short, the second covering greater portion of the upper surface, first segment and the front part of second segment with longitudinal striation, the rest of the abdomen smooth and shiny. Length 0·9 mm."

5. Lestodiplosis *sp.*

(*Itonididæ* : *Diptera*)

During the intensive study of the life-history of *Lixus truncatulus* in which several hundreds of eggs collected from field were carefully examined, in four cases a cecidomyid larva was found in association with the weevil egg (Plate XXVII, fig. 7). Two of these larvae were successfully bred to the adult stage and were identified by Barnes as a species of *Lestodiplosis*. In none of the four cases under observation did the weevil egg hatch out and in one instance an apparently empty egg case of the weevil was noticed after the pupation of the cecidomyid larva. Due to insufficient material a more thorough examination of the relationship of the two insects was not possible. It is therefore difficult to state with certainty its exact status, but it seems very likely that the larva was predaceous on the weevil egg. Dr. Barnes' views on this point expressed in his letter dated 1st January 1937 are as follows :—

" It is not a predatory form and so probably came from *Amarantus* not the *Lixus*. There are no previous records of gall midges of these genera being found on *Lixus* and *Amarantus*."

In view of the above remarks, it would appear to be a record of doubtful predatism but Barnes seems to have overlooked the fact that a species of *Lestodiplosis* is already known to parasitise the strawberry weevil, *Anthonomus signatus* in Minnesota [Marcovitch, 1917], while several species of *Lestodiplosis* are known to attack dipterous larvae and pupae including those of other cecidomyids [Kieffer, 1900].

The cecidomyid larva in the beginning of last stage measures 1·25 mm. long and 0·25 mm. broad. As in many other cecidomyids, the body is flat dorsoventrally, comparatively broad towards the posterior end, gradually tapering anteriorly, and bearing a pair of antennae. The three thoracic segments are as usual pink in colour and the sternal spatula situated ventrally on the first thoracic segment is rather small.

The pupa is about 1·25 mm. long and 0·5 mm. broad and possesses a general salmon-pink colouration. The abdomen is large and plump, the folded legs not going beyond the fifth abdominal segment. The head bears anteriorly a pair of spines, behind which are seen a pair of backwardly directed fine hairs. The thorax also carries a pair of spines.

The adult midge (Plate XXVII, fig. 8) is a little over 1·3 mm. long and 0·5 mm. broad. The antennae are long, thirteen segmented, each segment bearing a whorl of hairs at the base. The neck of all the segments is roughly of the same size and nearly three times as long as broad. Head is dark, the thorax pale brownish and the abdomen bright pink. The wings, which are 1·5 mm. long and a little over 0·6 mm. broad, are fringed with hairs. The third vein reaches just beyond the tip of the wing and fifth vein forked at about one third its distance from the tip.

6. Dinarmus sauteri *Masi*

(*Miscogasteridae* : *Chalcidoidea*)

This parasite was found parasitising to a small extent well-developed larvae of *Lixus truncatulus*. Masi [1926] described this species from specimens collected by Sauter from Formosa (Japan) in 1912. The writer has not been able to find any record of this species from any other part of the world since that date.

The grub is elongate and active. When full grown, it measures 2·7 to 2·8 mm. in length and 0·7 to 0·8 mm. in breadth. It pupates within the tunnel close to the remains of the host grub. The pupa is straight with rather small eyes, brownish antennae and a large abdomen. At 27°C. the pupal period is only four to five days.

Adult.—The original description of the species (op. cit.) is based on female specimens only. Masi has kindly sent me a revised translation of this which I reproduce below after slight modifications (Plate XXVII, fig. 10).

Female.—Length 4·5-5·0 mm. Aeneous-green; the abdomen above deep violet, except the basal tergite which is brassy-green; sometimes abdomen above violet-brown; eyes chestnut; antennae reddish yellow, ninth and tenth antennal segments (fourth and fifth of funicle) and sometimes the sixth (first of funicle) darker, also sometimes the 8th (third of funicle) testaceous and the following ones dark; the club always dark. Legs brownish, with ochraceous tarsi; knees, apices of mid tibiae, apical one third of posterior tibiae, whitish or yellowish, the darker colouration always sharply defined against the paler colour. Wings slightly greyish, veins dark brown.

Head shorter than long; antennae inserted above the ocular line in the middle of face; margin of the clypeus deeply incised at the middle; genal sulcus faint, orbits elliptical, vertex obtusely angulated and rounded. Scape reaching beyond the front ocellus; pedicel nearly as long as the three ring-joints combined; the third ring-joint one and a half times longer than the preceding one and a little broader; first funicular segment three times longer

than broad and twice the length of pedicel; the following segments becoming gradually shorter and broader, the 5th a trifle longer than half of the 1st, one and half times longer than broad; length of club equal to the first segment and the third ring-joint combined. Fore wings well rounded apically, ratio of the costal cell, marginal, post-marginal and stigmal veins 100 : 51 : 36 : 20; stigmal vein somewhat clavate, basal cell and mirror (naked area) naked, the remaining space covered with rather densely set setae; of these at least 30 between the stigmal and post-marginal veins (on the upper side of the wing) four or five in each transverse series. Dorsellum (central part of the true metanotum) with the usual two pits at its extremities; propodeum not carinated medially, densely punctate, with the posterior part distinctly bordered near the petiolar articulation; stigma twice longer than broad, separated from the median area of propodeum with two superposed pits, the lower of which is more visible and deeper. 'Mesepimerum' (morphologically inferior. part of mesepimerum) shining, seen with the microscope finely reticulate. Abdomen not longer than the rest of body, nearly equal to the thorax in breadth, very minutely reticulate.

As there is marked dimorphism in this species and the male has *not* been described so far, it has been considered necessary to figure both the sexes (Plate XXVIII, figs. 10 and 11) and to give below important points in which male differs from the female :—

Male.—Length about 2·4 mm. General colour bright metallic green. Excepting the apical third of first, the whole of the second, and the base of the third tergites which are white, the rest of abdomen very dark violet or dark coppery brown. Scape and pedicel light brown, rest of antennae dark brown. Coxae concolourous; fore and mid femora reddish brown; hind femora coppery brown; basal halves of tibiae light brown; all knees, apical halves of tibiae, tarsi except apical segment almost white. Veins relatively lighter than in the female. Antennae inserted well above the middle of face, ring-joints two, pedicel nearly equal to the first funicular segment. Propodeum with a distinct median carina, sculptured like the rest of thorax.

Allotype—1 male on a card, in the Pusa collection.

Paratype—1 male on a card, in the Pusa collection.

Pusa, T. Ahmad, ex grub of *Lixus truncatulus*, 9-12-1935.

7. Eurytoma curculionum *Mayr*

(*Eurytomidae* : *Chalcidoidea*)

The grubs of this parasite were found feeding on the grubs and pupae of *Lixus truncatulus* in small numbers. The parasite grub is a voracious feeder, measuring when full grown about 4 mm. long and 1-1·25 mm. broad. This species is known to parasitise grubs of *Cleopus campanulae* and *Gymnetron asellus* in Austria [Mayr, 1878], *Baris* spp. in France [Faure, 1923] and *Lixus junci* Boh., a pest of seed beet in central Italy [Menozzi, 1934; 1936].

In the latter case the parasite is considered to be of sufficient importance and attempts are being made to breed it artificially and liberate it for the control of *Lixus junci*.

The pupa (Plate XXVIII, fig. 8) has rather small, characteristically red eyes and brownish antennae on a small head. It measures 4 mm. long and 1·25 mm. broad. The thorax is prominently bulging out with distinct three segments. The folded legs do not go beyond three fourth the length of abdomen. The abdomen is plumpy, the length being only a little over one and a half times the breadth. The pupal period is eight to nine days at 27°C.

The adult parasite (Plate XXVIII, fig. 9) is a stout-looking black insect about 4 mm. long and 1·25 mm. broad. The entire body with the exception of eyes and legs are jet black. Eyes are deep brownish red. The tibiae and front tarsi are light brownish red. The femora are smoky black, while the middle and hind knees and tarsi are whitish excepting the terminal segments which are dark. The abdomen is elongate, about two and a half times as long as broad.

IV. Acknowledgments

I have great pleasure in recording my sincere thanks to Dr. H. S. Pruthi, Imperial Entomologist, for facilities and useful suggestions given during the work; to Mr. M. S. Mani, Assistant to Imperial Entomologist, for the identification of parasites in general and for the description of the male of *Dinarmus sauteri* in particular; to the Bureau of Entomology, United States Department of Agriculture for naming some parasites and to Mr. P. Narayanan, artist, for painting the plates illustrating the paper. Finally I must acknowledge gratefully the information furnished by the Entomologists, Madras, Dehra Dun and Indian Museum, Calcutta, regarding the distribution of *Lixus truncatulus* from the specimens of this species in their collections.

V. Summary

The Amarantus borer, *Lixus truncatulus*, is widely distributed throughout India, as a minor pest of several species of amarantus cultivated for vegetable, medicinal or ornamental purposes. The weevil has actually been bred from *Amarantus gangeticus*, *A. caudatus*, *A. viridis*, and *A. spinosus*. In case of scarcity of these food plants eggs are also laid after making several empty holes in *Chenopodium album*.

The adult weevils are almost mature on emergence. They copulate for seven to eight hours and begin egg-laying soon thereafter. Eggs are laid singly in holes bored in tender branches of plants, or in the petioles and midribs of leaves and covered over with a sticky secretion. The maximum number of eggs laid by a female during her life of about five months was 174. The incubation period is four days at 27°C. and ten days at 20°C. The grub stage lasts forty to fifty-five days during spring, while the grubs hatched in autumn

pass the entire winter in this stage. During winter the grubs can live without food for about three months. The pupal period is nine to ten days at 27°C. and twenty to twenty-four days at 20°C. The adult weevils normally live for four to six months. From March to November there are roughly three broods which overlap each other.

The life-history and morphology of five egg parasites, one larval parasite and one pupal parasite of the weevil are described. All these parasites have been bred from *Lixus truncatulus* for the first time from India. Of the egg parasites, *Pareuderus torymoides* Ferriere seems to be the most important ; it parasitises up to 31 per cent of eggs at Pusa. Two species of *Anastatus*, and *Telenomus javensis* Dodd come next and are of minor importance as egg parasites. A cecidomyid, *Lestodiplosis* sp., is also recorded as a predator on eggs of the weevil.

The chalcid parasite, *Dinarmus sauteri* Masi parasitises the grubs to a small extent. As there is marked dimorphism in this species and the original description is based on female specimens only, the male of the species is also described.

Lastly *Eurytoma curculeonum* Mayr is recorded as parasitising the grubs and pupae of the weevil in small numbers.

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THE BIONOMICS, LIFE-HISTORY AND CONTROL OF THE GRASSHOPPER *POECILOCERUS PICTUS* (FAB.)—A NEW PEST OF CULTIVATED CROPS IN NORTH INDIA

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(With Plates XXIX and XXX)

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I. Introduction

The grasshopper *Poecilocerus pictus* (Fab.) is quite familiar to the students of entomology and needs no special introduction. On account of its large size it is selected as a type for teaching insect morphology in some Agricultural Colleges. Though its anatomy has been studied in some detail, its bionomics, life-history, etc., have not been fully investigated. Obviously this is due to the fact that this grasshopper has not attracted the attention of economic entomologists since it is not considered to be a pest of any cultivated crop. It chiefly feeds on a wild plant called *ak* (Hindustani), a species of the genus *Calotropis*, and hence it is popularly called as the *ak* grasshopper. With

the exception of a record by Fletcher [1914] of this grasshopper having been found damaging young fig trees in South India, it was not known to cause damage to any plants of economic value until recently when in the summer of 1937 the writers noticed it causing considerable damage to brinjal, castor and tomato in a village garden at Shadipur near the estate of the Imperial Agricultural Research Institute. During April and May it was observed feeding on *ak*, but when it had almost exhausted this food plant it migrated to the neighbouring crops named above. It was thus realized that this grasshopper threatened to be a potential pest of cultivated crops and therefore it was considered advisable to study its biology, range of food plants, etc., and try some methods of control. The grasshopper has been closely studied during the past two years and the results of the investigations are reported in the following pages.

II. Distribution

This grasshopper is known to occur throughout the plains of India except in Bihar, Bengal and Assam. Outside India it is found in Africa. It appears that this grasshopper likes dry and hot climate.

III. Food plants and nature of damage

Poecilocerus pictus, as its popular name (*ak* grasshopper) indicates, is very fond of *ak* and feeds upon every part of it, *e.g.*, leaves, stems, pods, flowers, etc., often reducing the plants to naked stems, not even leaving the green bark behind. With a view to investigating the potentiality of this grasshopper as a pest of cultivated crops, experiments were carried out with numerous food plants available at Delhi during summer, when this insect is commonly met with in the field. In order to know whether the hoppers were capable of growing and developing on different food plants, they were confined in tall wire gauze cages with different kinds of leaves which were changed every morning. The following plants were tried :—brinjal, tomato, *bhindi* (*Hibiscus esculentus*), potato, gourds, cabbage, radish, musk-melon, castor, oleander, maize, cotton, broad bean, hollyhock, sorghum, cowpea, *pipal* (*Ficus religiosa*), tobacco and sugarcane.

Out of the plants named above, the hoppers fed readily on the leaves and fruits of brinjal and tomato, and on the leaves of *bhindi*, potato, gourds, cabbage, radish, musk-melons, castor, oleander and maize. They underwent normal moulting and became adults. The hoppers fed moderately on cotton, broad bean, hollyhock, sorghum and cowpea, and underwent moulting. The *pipal* leaves were only nibbled a little and sugarcane and tobacco leaves were not eaten at all.

In the field so long as *ak* plants were available, the grasshopper was mostly confined to them. Where brinjal plants were growing in the vicinity of *ak*, the hoppers were found infesting them but to a less extent. It appears that this insect becomes a menace to cultivated crops if its population becomes very large during spring when it is still on its wild food plant, *viz.*, *ak*.

IV. Life-history and description of immature stages

The life-history of *P. pictus* in broad outline is as follows :—

The eggs are laid in soil from June to August and sometimes even in September. The winter is generally passed in the egg stage and the young hoppers hatch out towards the end of March or beginning of April at Delhi. The nymphs feed and grow during April and May and become adults about the end of the latter month, after undergoing five or six moults. The males and females start copulating within one or two weeks of the final moult, and egg-laying and active breeding continues up to the end of September, by which time the last batch of eggs has been laid. After the middle of October hoppers begin to dwindle in numbers, but there are numerous egg masses in the soil during winter. Only a very insignificant percentage of the eggs laid in summer hatch in autumn but the great majority do not hatch till the ensuing spring, as stated above.

The life-history is described in detail below :—

The two sexes can be easily distinguished. The males are smaller and more slender than the females. The extremity of the abdomen in the male is conical and turned upwards, while in the female it ends in a number of paired chitinous hooks, which constitute the ovipositor.

Copulation

The male does not start copulating immediately after it becomes adult. The pre-copulation period in this sex varies from five to eight days. However, the female is ready for copulation soon after it undergoes the final moult. On several occasions fresh adult females were given to sexually mature males and the copulation started almost immediately. The process of copulation is almost the same as in other grasshoppers. It may last from two to four, sometimes even six hours and in rare cases even one day. The males as well as females were found to copulate several times, which may be as many as fifteen times within twenty to twenty-five days, and both polyandry and polygamy are quite common. There is frequent copulation both before and after oviposition.

Oviposition

The egg-laying begins from twenty to twenty six days after the emergence of the female as adult. The period intervening between the first copulation and the first egg-laying varies from fourteen to twenty days.

The ovaries of females of different ages were dissected to note the time of maturity of eggs. It was found that in the case of the females which had copulated, the eggs were mature after fifteen or sixteen days, while in the ovaries of the virgin females the eggs were found to be fully mature after twenty two to twenty four days. The pre-oviposition period in the virgin females was found to be twenty four to twenty seven days. Observations on the pre-oviposition periods and the number of ovipositions and eggs laid within a period of two months of adult life have been summarized in Table I.

TABLE I

Pre-oviposition period and number of ovipositions and number of eggs laid

Serial No.	Date of attaining adult stage	Date of first oviposition	Pre-oviposition period (days)	Date of subsequent oviposition	No. of egg masses laid	No. of eggs
1	11th June 1938	3rd July 1938 .	23	21st July 1938 .	2	Kept in soil to note hatching
2	13th ,, .	5th ,, .	23	22nd ,, .	2	
3	14th ,, .	18th ,, . (found after some time).	35	...	1	120
4	15th ,, .	4th ,, .	20	...	1	40 (on the surface)
5	14th ,, .	5th ,, .	22	12th July 1938 .	2	...
6	14th ,, .	5th ,, .	22	...	1	110
7	12th ,, .	...	...	18th July 1938 .	2	...
8	14th ,, .	5th July 1938 .	22	...	1+1	108
9	14th ,, .	5th ,, .	22	12th July 1938 .	2	...
10	17th ,, .	8th ,, .	22	24th ,, .	1+1	108
11	16th ,, .	5th ,, .	20	...	1	77
12	16th ,, .	7th ,, .	22	...	1	...
13	18th ,, .	14th ,, .	27	...	1	...
14	19th ,, .	13th ,, .	25	...	1	...
15	19th ,, .	14th ,, .	26	...	1	...
16	19th ,, .	21st ,, .	33	...	1	...
17	15th ,, .	6th ,, .	22	...	1	...
18	15th ,, .	8th ,, .	24	...	1	133
	15th ,, .	9th ,, .	25	...	1	120
20	17th ,, .	6th ,, .	20—21	...	1	117
21	17th ,, .	8th ,, .	22	...	1	105
22	16th ,, .	7th Nov. 1938 .	22	...	1	...
23	16th ,, .	5th July 1938 .	20	...	1	65
24	17th ,, .	6th ,, .	20	22nd July 1938 .	2	105
25	17th ,, .	17th ,, .	31	...	1	98 (on wood)
26	15th ,, .	8th ,, .	24	26th July 1938 .	2	...
27	14th ,, .	5th ,, .	22	21st ,, .	2	...
28	11th ,, .	4th ,, .	24	13th ,, .	3	...
				28th ,, .	...	...
		Average .	23	...	1·4	116
			Virgin females.			
1	17th June 1938 .	12th July 1938 .	26	...	1	3 or 4
2	17th ,, .	10th ,, .	24	13th July 1938 .	2	...
3	17th ,, .	11th ,, .	25	...	1	102
4	29th ,, .	22nd ,, .	24	...	1	...
5	19th ,, .	17th ,, .	29	...	1	...
6	17th ,, .	...	...	...	1	140
		Average .	25 days	...	...	...

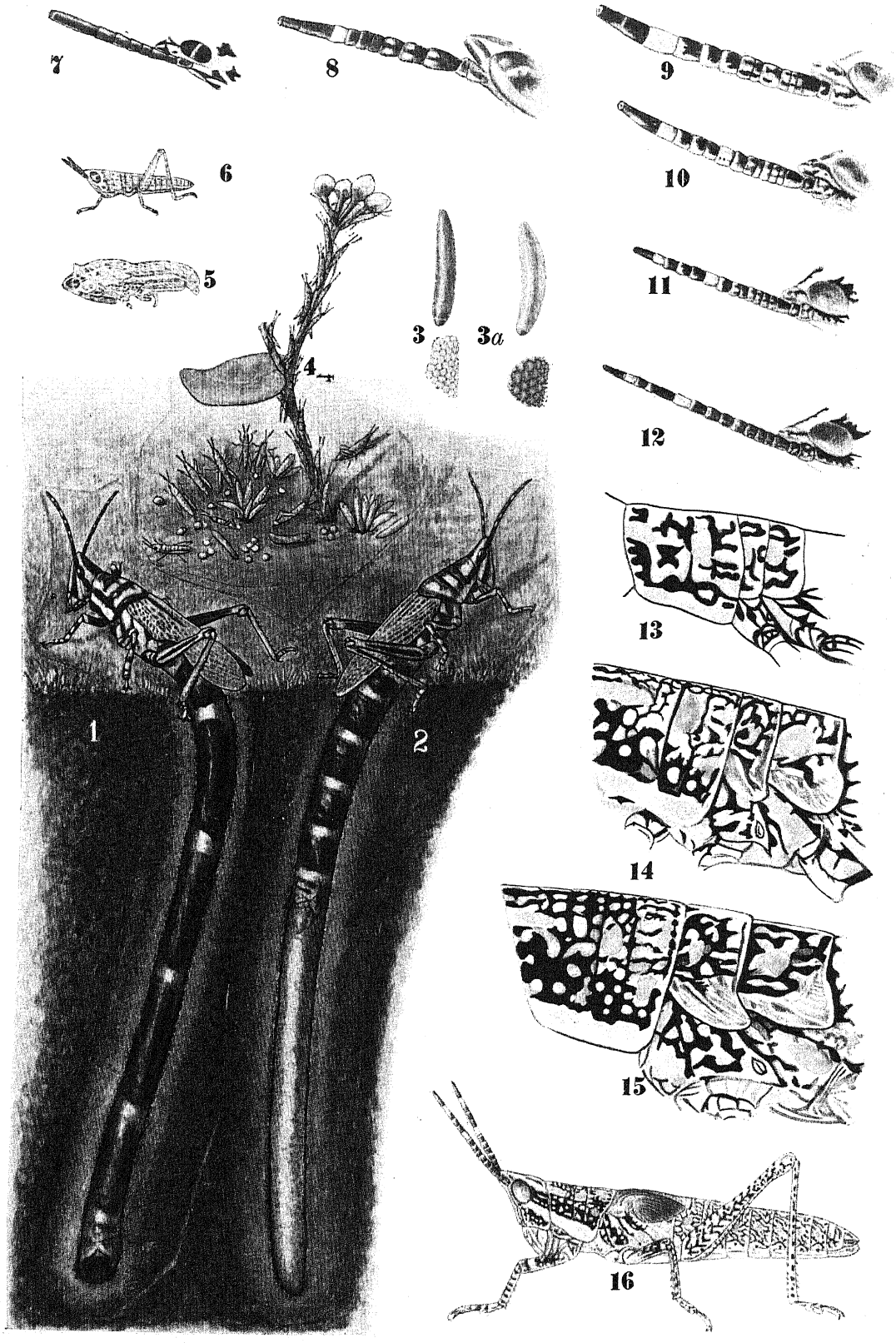
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PLATE XXIX

FIGS. 1 & 2. *Poecilocerus pictus*, females, in the process of egg laying. Note that the abdomen has been extended upto the length of about 5 in. (Fig. 1). In Fig. 2. the abdomen is being retracted after eggs have been laid, ($\times\frac{1}{2}$)

FIG. 3. Freshly laid egg ($\times 2\frac{1}{2}$), with a part of the chorion highly magnified

FIG. 3*a*. The egg about to hatch ($\times 2\frac{1}{2}$), with a part of the chorion highly magnified

FIG. 4. Freshly hatched young hoppers. Natural size

FIG. 5. Young 'larva' ($\times 2\frac{1}{2}$)

FIG. 6. 1st instar nymph ($\times 2\frac{1}{2}$)

FIGS. 7—12. Antenna and compound eye of the I—VI instar nymphs

I	×10
II—III	×8
IV	×5
V—VI	×3

FIG. 13. Lateral view of thorax of the 1st instar nymph (×10)

FIG. 14. Lateral view of thorax of the 3rd instar nymph (×8)

FIG. 15. Lateral view of thorax of the 5th instar nymph (×5)

FIG. 16. Sixth instar nymph ($\times 1\frac{1}{2}$)

PLATE XXIX

Figs. 1 & 2. *Poecilocerus pictus*, females, in the process of egg laying. Note that the abdomen has been extended upto the length of about 5 in. (Fig. 1). In Fig. 2. the abdomen is being retracted after eggs have been laid, ($\times\frac{2}{3}$)

Fig. 3. Freshly laid egg ($\times 2\frac{1}{2}$), with a part of the chorion highly magnified

Fig. 3a. The egg about to hatch ($\times 2\frac{1}{2}$), with a part of the chorion highly magnified

Fig. 4. Freshly hatched young hopper. Natural size

Fig. 5. Young 'larva' ($\times 2\frac{1}{2}$)

Fig. 6. 1st instar nymph ($\times 2\frac{1}{2}$)

Figs. 7—12. Antenna and compound eye of the I—VI instar nymphs

I	$\times 10$
II—III	$\times 8$
IV	$\times 5$
V—VI	$\times 3$

Fig. 13. Lateral view of thorax of the 1st instar nymph ($\times 10$)

Fig. 14. Lateral view of thorax of the 3rd instar nymph ($\times 8$)

Fig. 15. Lateral view of thorax of the 5th instar nymph ($\times 5$)

Fig. 16. Sixth instar nymph ($\times 1\frac{1}{2}$)

The eggs are laid in the soil about 5-6 in. below the surface. Generally the soil which is selected for this purpose is of loose texture, hence more easily penetrable. The gravid females have inflated abdomens. When ready for egg-laying, the female trails its abdomen on the ground searching for a suitable spot by scratching the surface of the soil with its ovipositor. When such a spot has been found it starts making an egg chamber with the help of the anterior and dorsal valves of the ovipositor. The valves act like the prongs of a forceps. The egg-laying process has been studied in the laboratory in a large glass cage (1-5 ft. × 1 ft. × 1 ft.) which was half filled with soil. The top of the soil was covered with a thick card board leaving the soil uncovered near the sides only. The female was thus forced to bore holes in close proximity to the glass sides and thus the whole process could be easily watched. During the course of boring, the extremity of the abdomen rotates through an angle of 180°, pressing and making compact all sides of the burrow. The formation of the egg chamber takes from one to two hours. The chambers are generally 6-6½ in. deep. Though the abdomen is only 1·4 in. long in the normal position, it can be extended to reach the depth of chamber with the help of the elastic intersegmental membranes (Plate XXIX, fig. 1). In many cases the female makes several chambers and discards them without laying any eggs therein probably because of the unsuitable texture or moisture content of the soil. Sometimes the female turns its abdomen in a zigzag fashion if it meets with any obstacle inside the egg chamber. Before the eggs are actually laid the female exudes a fluid which is worked up into a frothy mass by the quick motions of the ovipositor valves and is deposited at the bottom of the egg chamber about 2 in. thick on which the eggs are laid one by one in a spiral manner so as to form a compact mass (Plate XXIX, fig. 2). The female gradually retracts its abdomen as the chamber gets filled up with eggs. When all the eggs have been laid, the vacant space in the chamber is filled with froth up to the top. The whole process takes about two to six and a half hours.

The female looks somewhat sluggish for a short time after oviposition. Generally one or two and sometimes three egg masses were laid by a single female during its life time under laboratory conditions. The unfertilized females generally laid one egg mass only. The number of eggs in a mass varied between 60 and 140, the average being about 116. It is likely that more than two egg masses are laid in nature.

Egg

The egg (Plate XXIX, fig. 3) is elongated, curved, almost cylindrical, thick in the middle and tapering towards the ends, which are bluntly rounded. When freshly laid, the egg is yellow but soon turns brown. The micropyle is darker in colour and can be easily demarked. When mature, the egg grows in thickness (Plate XXIX, fig. 3 *a*). There are two egg coverings, the chorion and the vitelline membrane. The former is sculptured and in a mature egg can be peeled off easily by scratching. The vitelline membrane is yellow, smooth but tough and cannot be pierced when pressed with an ordinary needle. When the egg is about to hatch, the young one inside can be seen through the

egg skin, moving its abdomen and striped hind femora. Just prior to hatching some black spots make their appearance on the surface of the chorion near the operculum. From these spots some transparent liquid oozes out, and it is at these spots that the membranes rupture and the 'vermiform larva' hatches out. Its head and antennæ (Plate XXIX, fig. 5) are bent downwards and the legs are folded. These "larvæ" make their way up through the dry froth by wriggling motions of their bodies and emerge out of the burrow in large numbers and present a very interesting appearance (Plate XXIX, fig. 4). The emergence of all the 'larvæ' from a single egg mass takes about ten minutes. Soon after reaching the surface of the soil, the 'larvæ' start the process of casting their covers, known as the intermediate moult. Just before this moulting, the cervical ampulla gets inflated and presents a two-knobbed swelling. By the alternate swelling and contraction of this ampulla the thin white skin is ruptured dorsally in the thoracic region and eventually the young nymph crawls out. The process of the intermediate moulting is completed within two to four minutes. Some previous authors have not considered this moult to be true one, but we, like Uvarov [1928], do not find any important difference between this and the subsequent moults.

The newly emerged nymphs are negatively geotropic, always trying to move upwards and ascending on any piece of stick or twig. They move in a mass towards the source of light and sit in clusters on any standing twig with their heads pointing upward (Plate XXIX, fig. 4). The nymphs begin to feed about twenty four hours after their emergence.

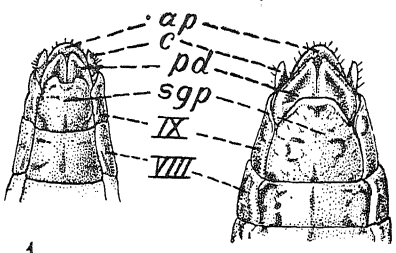
There are usually six nymphal moults before the individual reaches the adult stage. Sometimes there are five moults only. The duration of the various instars is variable. On an average (200 observations) they are as follows during summer :—

1st instar	9·7 days.
2nd ,,	7·8 ,,
3rd ,,	7·8 ,,
4th ,,	8·5 ,,
5th ,,	9·9 ,,
6th ,,	11·6 ,,

The total nymphal life lasts for fifty to fifty-six days during summer. In the case of those few eggs which hatch in autumn, the nymphal period is very long. Only a few of these nymphs become adults in November while the great majority of them over-winter and become adults in the following April after a nymphal life lasting seven or eight months.

Different Nymphal stages

First stage nymph.—The freshly emerged nymphs (Plate XXIX, fig. 6) are orange coloured, with white and faint blackish stripes. After the lapse of one day the blackish patterns become dark and the hoppers look yellowish grey in general appearance. They measure 9-12 mm. in length and about 2·5 mm. in breadth (in the region of the thorax). The pronotum is about 1·8 mm. long and the antenna about 2·25 mm.



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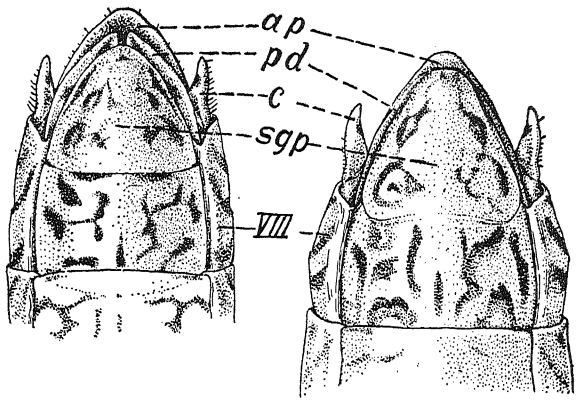

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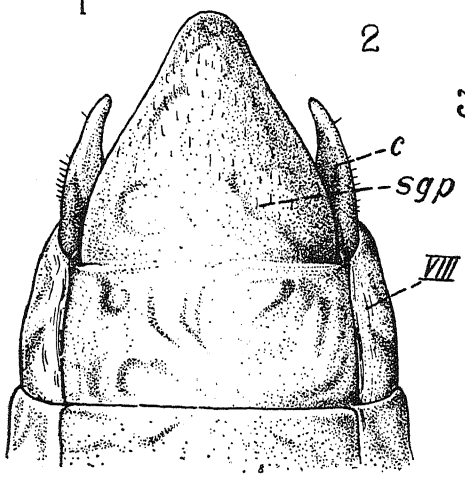

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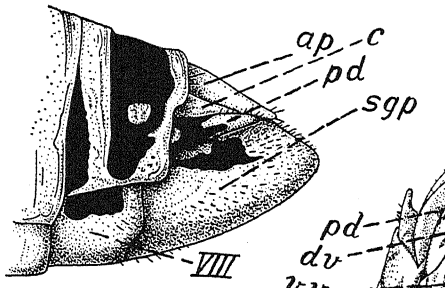

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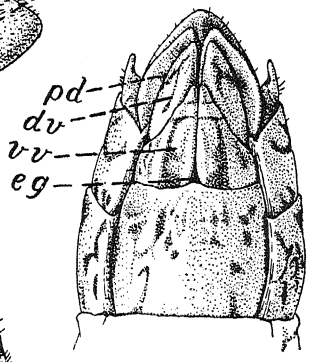

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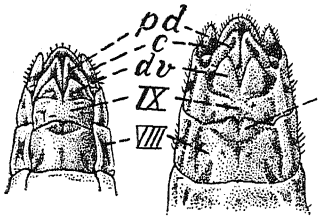

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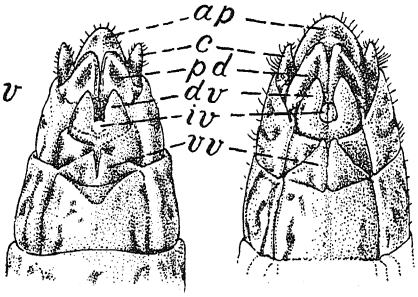

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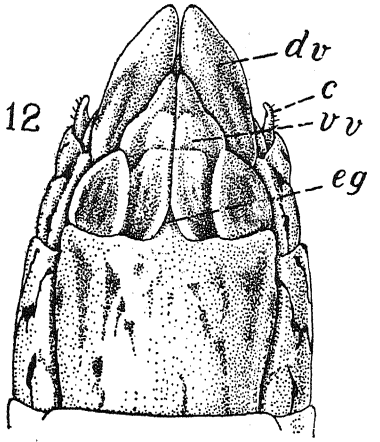

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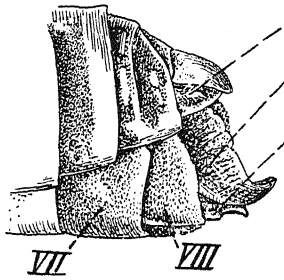

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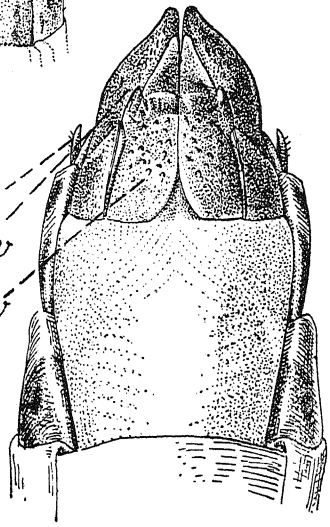

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PLATE XXX

FIG. 1—4 Posterior abdominal segments of the male nymphs of 1st, 3rd, 5th, and 6th instars respectively ; ventral view ; ×10

FIG. 5 Posterior abdominal segments of the male adult grasshopper ; ventral view ; ×10

FIG. 6 The same in lateral view ; ×7

FIGS. 7—12 Posterior abdominal segments of the female nymphs of 1st to 6th instars respectively ; ventral view ; ×10

FIGS. 13—14 Posterior abdominal segments of the adult female grasshopper ; lateral and ventral views respectively ; ×7

LEGEND TO LETTERING

ap—anal plate ; *c*—cerci ; *dv*—dorsal valves of the evipositor ; *eg*—egg guide ; *iv*—inner valves of the ovipositor ; *pd*—podical plates ; *sgp*—subgenital plate of the male ; *vv*—ventral valves of the ovipositor ; VII-VIII—seventh and eighth abdominal segments.

PLATE XXX

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LEGEND TO LETTERING

ap—anal plate; c—cerci; dv—dorsal valves of the ovipositor; eg—egg guide; iv—inner valves of the ovipositor; pd—podical plates; spp—subgenital plate of the male; vv—ventral valves of the ovipositor; VII-VIII—seventh and eighth abdominal segments.

The head is conical and sloping in the front view. It projects forward between the compound eyes and forms a triangular area known as the fastigium. The margins of the fastigium are slightly raised. The eyes are oval, black and devoid of any stripe. The frons is traversed by a deeply sulcate frontal ridge, running from the tip of the fastigium up to the clypeus. The margins of the ridge—the frontal carina—are whitish in colour, and are bordered on each side by two yellow or orange patches.

The antennæ (Plate XXIX, fig. 7) are deep purple and are invariably eightsegmented. The first or the basal joint is cylindrical and the second is small and globular. The third to seventh segments are almost subequal. The eighth segment is very long and is equal in length to the three preceding segments taken together.

The pronotum is saddle shaped and has a very feeble medial carina. There are five yellow patches on each lateral lobe, the arrangement of which is constant as shown in the figure (Plate XXIX, fig. 13). The prosternum bears a very acute compressed aciculate tubercle, which is situated between the two coxæ. The mesonotum is a small narrow sclerite partly hidden below the pronotum. The metanotum resembles mesonotum except that it is broader than the latter. The metapleuræ have each a long oblique orange patch, in front of which are the small, almost circular spiracles (*sp.*) which are externally provided with two-lobed valves. The first pair of spiracles remain hidden under the pronotum.

The first and second pair of legs are small and have black and white markings all over. The third pair is stout. The upper and lower ridges of the hind femora are traversed with eight to nine and five to six black dots respectively. The hind tarsi are three-jointed, the terminal joint being provided with a pair of claws and a median pad, the arolium.

The abdomen is almost cylindrical. It has a mid-dorsal stripe of white spots interrupted with black markings. On each side of this stripe there is a lateral interrupted stripe of orange spots. Ventrally the abdomen is silver grey, with black lines interspersed all over. In both sexes ten terga are distinct, the last two being partially fused. The eleventh tergum and the supra-anal plate are fused into one piece at the sides of which and slightly lower in level are the two small cerci, the appendages of the eleventh abdominal segment. The number of apparent sterna in the two sexes are different. In the male there are ten sterna including the first one which is fused with the sternum of the metathorax. The ninth sternum is large, slightly depressed ventrally and deeply notched at the posterior margin ; this is the rudiment of the subgenital plate (Plate XXX, fig. 1, *sgp*). Above this plate there is a pair of triangular outgrowths known as the podical plates (*pd*). In the female also ten sterna are visible, the ninth being cleft into two triangular pieces, which are the rudiments of the dorsal valves of the ovipositor (Plate XXX, fig. 6, *dv*). Lying above the pieces there is a pair of triangular podical plates (*pd*) as in the male. At the posterior margin of the eighth sternum there is a very tiny pair of protruberances which are the rudiments of the ventral valves of the ovipositor (*vv*)

It is not easy to distinguish sexes at this stage as the rudimentary subgenital plate of the male which is notched at the posterior margin, and the ninth sternum of the female which is cleft into two parts, looks very much like. But the presence of the rudimentary ventral valves of the ovipositor, resent at the posterior margin of the eighth sternum, distinguishes the emale from the male (Plate XXX, *cf.* figs. 1 and 7).

Second stage nymph.—The second stage nymph resembles in general appearance and colour the first stage nymph, but is bigger in size, measuring 10-13·5 mm. in length and 2·5 mm. across the pronotum. The antennæ are 3·0 mm. long and are usually nine-jointed (Plate XXIX, fig. 8), the sixth joint of the first stage having divided into two. Sometimes the antennæ consist of eight segments only. On the distal margin of the third to sixth joints there are two small white spots which are totally absent in the first stage. The dark compound eyes develop each a transverse whitish stripe.

There is no indication of the wing pads in this stage but the posterior angles of the meso and meta-nota become somewhat extended outwards getting thinner and slightly dark in colour.

There is no change in the abdomen except in the appearance of some new yellow patches, the broadening of the black patterns, and the further development of the external genitalia.

The ninth sternum or the subgenital plate of the male grows in size and the notch begins to flatten out. In the females the ventral valves and dorsal valves of the ovipositor grow longer and gradually extend backwards and cover part of the ninth segment and anterior half of the podical plates respectively (Plate XXX, fig. 8).

In this stage spiracles are visible on the lateral folds of the first eight abdominal segments, the first pair of spiracle being near the tympanum.

Third stage nymph.—The general colour of the body becomes deeper in this stage. The median yellow spots on the dorsal side of the abdomen turn orange in colour. The length of the body is 15-17 mm.

The antennæ (Plate XXIX, fig. 9) in this stage are about 4 mm. long and are ten or eleven jointed, the fifth joint and sometimes sixth as well of the previous stage having each been divided into two segments. The tenth or the last segment is dull purple in the basal half and light purple in the distal region, and can therefore be mistaken for two joints.

The pronotum measures 2·7 mm. The meso and meta-nota develop distinct, thin triangular outgrowths at their postero-lateral angles. These outgrowths are the rudiments of the future wings (Plate XXIX, fig. 14, *wp.*).

The subgenital plate of the male completely loses its notched appearance and becomes rounded at the posterior margin (Plate XXX, fig. 2). In the female the eighth segment develops a crescent shaped fold (*eg*) directed inwards near the bases of the ventral valves which now cover the base of the dorsal valves. This fold is the rudiment of the egg guide which becomes distinct in the fifth stage. A small transverse pad-like growth (*iv*) appears in this stage also between the bases of the dorsal valves (Plate XXX, fig. 9).

Fourth stage nymph.—The general colouration becomes still deeper in this stage. The body measures 20-24 mm. long and 3-4 mm. broad. The eyes are light brown near the lateral carina of the front, and their transverse stripes become broader than in the previous instar. There are twelve to fourteen joints in the antennæ which are now 5·6 mm. long (Plate XXIX, fig. 10). The third and fourth segments show an incomplete division, while the fifth segment of the previous instar has divided into two segments.

The pronotum measures 3·2 mm. in length. The postero-lateral angles of the meso-notum and meta-notum grow thin and dark and acquire oblique folds and thus form the rudiments of wing pads (Plate XXIX, fig. 15). Further, in some nymphs towards the end of this stage the wing pads begin to turn upwards, and the mesonotal pair comes to lie below the metanotal pair. However, in great majority of the nymphs the wing pads turn in the fifth stage.

In the male, the subgenital plate becomes conical and spatula-like in appearance. The podical plates get gradually pushed up and become compressed due to the development of the subgenital plate. In the female (Plate XXX, fig. 10) the ventral valves extend further backwards and their posterior ends become narrow and pointed. The dorsal valves also grow bigger, the transverse pad at their base develops a longitudinal fissure resulting in two minute appendages (*iv*), which are the rudiments of the inner pair of valves of the ovipositor.

Fifth stage nymph.—The general colouration of the body develops a shining yellow tinge in the places where there were yellow and white patches in the previous stages. The orange coloured spots grow deeper in tinge. The size of the hopper increases, being about 25-27 mm. in length and 4·5-5 mm. in breadth. The antennæ (Plate XXIX, fig. 11) are fifteen-jointed and measure 7-8 mm. The third to tenth segments are laterally compressed and rhomboid in cross section ; those from eleven to fifteen are more or less cylindrical.

The anterior margin of the pronotum remains straight as in the previous stages but the posterior margin becomes convex at the middle. The wing pads get upturned in this stage, if they had not done so in the previous stage, and develop numerous unbranched veins. In the male, the subgenital plate reaches the supra anal plate (Plate XXX, fig. 3). In the female the ventral valves grow thicker and extend very much backwards, reaching the middle of the dorsal valves. The latter also grow and extend up to the tips of the podical plates. The egg guide (*eg*) is bigger and distinct (Plate XXX, fig. 11).

Sixth stage nymph.—The freshly moulted nymph of this stage (Plate XXIX, fig. 16) is lemon yellow in general colour with black markings. All the white patches of the previous stage become yellow and the black patches grow deeper in tinge. It measures about 35-43 mm. in length and 7·5 mm. in breadth. The description of this instar relates to those specimens which turn their wing pads in the fifth instar. It is noteworthy that the upturned state of wing-pads last in every case for two stages only, that is to say, the nymphs which turn their wings in the fourth stage become adult at the end of

the fifth stage after undergoing five moults only, and those which turn their wing-pads in the fifth stage become adult at the end of the sixth stage. The latter condition is more common than the former.

In this stage the eyes turn slightly bluish in colour and their transverse stripes disappear altogether. The antennæ measure 11-13·5 mm. and have fifteen or sixteen joints. The posterior margin of the pronotum grows backwards and covers the bases of the wing-pads, which in this stage are larger, thicker and dark blue in colour.

In the male, the subgenital plate becomes a conical, pouch-like organ into which the ædeagus is lodged. This plate extends beyond the extremities of the podical plates, hiding them from the ventral view, the margins of the supra anal plate being only visible (Plate XXX, fig. 4). In the female, the egg guide becomes fully developed and remains pushed in between the two ventral valves. The dorsal valves grow very thick and extend far beyond the podical plates. They develop a lateral fold externally. The ventral valves develop a transverse fold in the basal region and a vertical fold in each of the lateral lobes and thus appear to be made of several pieces (Plate XXX, fig. 11).

Just before the nymph is about to moult finally for attaining the adult stage its colour and different parts of the body undergo very well marked changes which can be easily made out through the old skin. All the black patterns on the body begin to turn bluish and all the orange and yellow spots fade away and instead continuous yellow patches on the blue background begin to appear. The basal region of the antennæ and the frontal ridge become bluish. The anterior half of the pronotum gets constricted, whereas the posterior half becomes broadened and blue in colour. The second pair of wing-pads begin to show a reddish tinge.

The ventral and dorsal valves of the ovipositor become highly chitinous. The tips of the latter curve upwards and their upper surface becomes broadly dentate. Each ventral valve develops a lateral tooth-like process in posterior region and a number of minute spines in the basal region. The lateral lobes of the ventral valves become very large and appear as distinct sclerities (Plate XXX, figs. 12 and 12-A.).

The final moult.—The process of final moulting is very interesting . The hopper, when about to moult, stops feeding and rests on a twig. It begins to contract and expand the anterior region of its body. The head is moved forwards and backwards and the pronotum is moved up and down by contraction of the cervical ampulla, causing the rupture of the skin along the medial line above the pronotum. This fissure gradually increases extending from the fastigium up to the wing-pads. The head and thorax begin to be gradually drawn out of the skin through the slit. The upturned wing-pads become horizontal in position and are drawn out of their sacs. The first and second pair of legs are then gradually drawn out. The third pair of leg is the last to come out. The cast skin is gradually left behind as the adult grasshopper draws itself out. During the whole process of moulting the hopper breaths hard and

keeps on expanding and contracting its body. Just after the moult the fore-wings are only partly expanded and the hind pair is folded like a fan. Gradually the wings are fluttered and raised vertically and within twenty to twenty-five minutes the elytra (fore wings) become fully expanded and develop bluish green colour with yellow veins and reticulations. The hind wings are hyaline to begin with but gradually develop a pink colour at the apex, which ultimately deepens into a red rosy colour. The insect then rests on the twig generally facing sunlight. A few minutes after, it starts feeding.

V. Control measures

Sprays

With a view to finding out some suitable insecticide for controlling this pest a number of stomach poisons were tried in the form of sprays, dusts and poison baits. Lead arsenate which is commonly used for such insects was not found effective either in the field or in the laboratory when sprayed at the usual concentration of 2 lbs. per 100 gallons of water. It did not kill any hoppers within ten to thirteen days. Between thirteen and sixteen days the mortality was 25 to 40 per cent. The hoppers were kept under observation in this and the following experiments for about sixteen days only because it was considered that if the poison would not kill the hoppers within a fortnight it would not have any practical utility.

In the control cage all hoppers were found living beyond sixteen days. The lead arsenite spray was prepared by dissolving 5 lbs. of stone-lime in 100 gallons of water to which 2 lbs. of powdered lead arsenate and 10 lbs. of *gur* were added and the whole mixture shaken thoroughly.

Sodium arsenite.—Sodium arsenite, which is a poison soluble in water, was found more effective. It was sprayed in laboratory cages at the rates of 2 lbs., 1 lb., and $\frac{1}{2}$ lb. per 100 gallons of water. With the dose of 2 lbs., 96 per cent of the hoppers were killed in eleven days, death starting within two days of feeding, and the average longevity period being six days. This dose was however found to be injurious to the sprayed plants and hoppers did not feed readily on them. With the dose of 1 lb., the mortality was 100 per cent in thirteen days, the death starting within three days of feeding and the average life being 8·5 days. This dose also proved harmful to the leaves and the hoppers did not feed well on the sprayed food. With the dose of $\frac{1}{2}$ lb. per 100 gallons, the leaves were not at all injured, but the rate of mortality was rather slow, being 90 per cent in twenty days, death starting within five days of feeding, and the average life being thirteen days. The hoppers fed moderately on the treated food. In the control cages all hoppers were alive after twenty days.

The above doses were repeated in the field on brinjal, castor, *jowar*, cucurbits, etc. The doses of 2 lbs. and 1 lb. per 100 gallons proved injurious to the leaves which moreover became unpalatable to the hoppers. The latter were present on the sprayed plants in less numbers on the next day. On the third or fourth day the treated plants were entirely devoid of hoppers

but a search for the dead ones did not reveal any carcases. With a dose of ½ lb per 100 gallons, there was no leaf injury. The hoppers were found feeding on the treated plants for a few days but gradually declined in numbers. However, the dead hoppers could not be traced easily, only one or two stray dead specimens being found under the plants here and there.

The sodium arsenite spray was prepared by dissolving 5 lbs. of lime and 1¼ lbs. of *gur* in 100 gallons of water and adding 2 lbs. of the poison. The spray was diluted twice or four times respectively when one lb. or ½ lb. doses were tried.

Calcium arsenite.—Calcium arsenite being insoluble in water does not affect the leaf tissue like sodium arsenite. It was used at the strengths of 2 lbs. and 1 lb. per 100 gallons of water. Both the strengths proved harmless to the leaves of *Calotropis*, cotton and oleander.

With a dose of 2 lbs., 95 per cent of the hoppers, feeding daily on the poisoned food, died in seven days in the laboratory cages, death starting within three days of spraying and the average life being five days. The hoppers however did not feed well on the sprayed food. Under field conditions 60 per cent of the hoppers feeding on the sprayed plants died within nine days, death starting within four days of feeding and the average life being 5·5 days. These hoppers had been allowed to remain on the sprayed plants in the field for thirty-six hours and then removed and put in the laboratory cages containing untreated food. The hoppers in the laboratory experiments were fed on sprayed food every day.

With a dose of 1 lb., the kill was 90 per cent in six days in the laboratory, the death starting within three days of feeding and the average life being four days.

Oleander plants were sprayed in the field with the above doses. The results were quite encouraging. The hoppers which had fed for forty hours on the sprayed plants, were removed and caged with sprayed food and about 90 per cent of them died in five days.

Dusting experiments

Sodium arsenite.—*Calotropis* and castor plants were dusted in the field with sodium arsenite mixed with lime in the proportion 1 : 4. The hoppers were collected after twenty-four hours of dusting and caged. From the treated plants only 33 per cent of hoppers died in six days, and the rest continued to live beyond sixteen days. They started dying on the fourth day after dusting. The leaves of *Calotropis* seemed more or less unaffected but those of castor plant showed a scorching effect.

Calcium arsenite.—Calcium arsenite dusted in the same proportion showed slightly better results. The leaves were not injured and about 50 per cent of hoppers died in five days. The remaining lived beyond sixteen days.

Paris green.—Paris green was mixed with fine wood ashes in the proportion of 1 : 8 and 1 : 16, and dusted over the *Calotropis* and oleander plants. Hoppers were allowed to feed for twenty-four hours on the dusted plant.

With a dose of 1 : 8, 60 per cent of the hoppers died in ten days, the remaining living beyond sixteen days. Hoppers started dying on the third day after dusting.

Sodium fluosilicate and lime in the proportion of 1 : 4 were similarly dusted on brinjal plants but very few hoppers were killed.

Baits

Sodium fluosilicate.—This bait was prepared in the usual way. One lb. of finely powdered sodium fluosilicate was mixed thoroughly with 20 lbs. of wheat bran ; 1 lb. of *gur* or 1 pint of molasses was dissolved in a small quantity of water and added to the bran mixture so as to make it into a crumbly mass. Some amyl acetate was added to give the bait a good smell of ripe banana fruits. The bait was scattered in the hopper-infested field but did not prove effective. The hoppers, which, as already described, seldom leave the plant and come down on the soil, did not take to the bait scattered on the ground. The hoppers kept in cages in the laboratory also did not feed on the bait.

Conclusion

Experiments described above show that the spraying of infested plants with calcium or sodium arsenite at the rate of 1 lb. and $\frac{1}{2}$ lb. per 100 gallons of water respectively seems to be the most satisfactory method of control.

VI. Summary

The grasshopper *Poecilocerus pictus* has been known to feed mostly on *ak* plant (*Calotropis* sp.), which is hardly of any economic value, but in the summer of 1937 it was found attacking brinjal and castor in a village garden near the estate of the Imperial Agricultural Research Institute.

Numerous food plants of economic value were tested in 1937 and 1938 to determine the potentialities of this new pest of cultivated crops. It is found that this grasshopper readily feeds on brinjal (leaves and fruits), tomato, castor, *bhindi*, oleandar, etc.

The seasonal-and life-histories and general bionomics of the grasshopper have been studied. The hoppers undergo five or six moults before becoming adult. The various instars have been described.

Several stomach poisons have been tested as sprays, dusts and baits. Spraying with sodium arsenite at the rate of half pound per 100 gallons of water has proved to be the most satisfactory method of control. Sodium fluosilicate bait, which is usually effective against surface grasshoppers, has not been found satisfactory due to the peculiar habits of the grasshopper.

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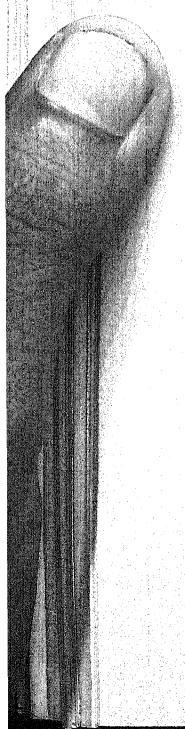

A NOTE ON THE DETERMINATION OF NITRATES IN WATERS AND SEWAGE

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INTRODUCTION

DURING the course of the analysis of sewage and effluents from the Activated Sludge Plant, Central Farm, Coimbatore, considerable difficulty was experienced in the determination of nitrates, especially in presence of large amounts of nitrites and soluble organic matter. The phenoldisulphonic acid method of determining nitrates in waters is the one most used and has been found reliable and quick, but owing to the presence of appreciable amounts of nitrites in the effluents, it was considered necessary to oxidise the nitrites beforehand either by potassium permanganate ($KMnO_4$) or by hydrogen peroxide (H_2O_2) as recommended in the standard methods [1925] of water analysis. In certain preliminary experiments where potassium permanganate was used as the oxidising agent considerable loss of nitrate was found to occur ranging from 35 to 90 per cent. This was rather surprising and a detailed study of the method was made to find out the causes of such loss.

EXPERIMENTAL

Experiment 1.

To trace the defects, if any, in the technique and procedure adopted for the determination of nitrates an experiment was conducted using dilute potassium permanganate for the oxidation of the effluents under different conditions and treatments.

Treatment.—(1) Neutralisation of the clarified effluent with dilute sulphuric acid ($N/10$) before adding neutral potassium permanganate and removal of the chlorides by silver sulphate;

(2) Oxidation of the clarified sample with acid potassium permanganate till the pink colour persisted, followed by neutralisation with $N/10$ sulphuric acid and removal of chlorides by silver sulphate ;

(3) Addition of neutral potassium permanganate to the raw sample before neutralisation, clarification and removal of chlorides ;

(4) Oxidation of the raw sample with acid potassium permanganate and determination of the total nitrates by Harper's method [1924].

The above experiment under each treatment was carried out in duplicate avoiding excess of silver sulphate in all the cases and the results obtained are recorded in Table I. It is surprising to note from the table that in all the treatments, the total nitrate values obtained are quite erratic and are far below the actual nitrites present in the samples.

TABLE I

Nitrite—N and total N (nitrite and nitrate) in the samples under different treatments

Treatment No.	Nitrite—N (parts per 100,000)	Average of two estimations		Per cent decrease of total N over nitrite—N
		Total N (NO_3+NO_2) after oxidation (parts per 100,000)	Decrease of total N over nitrite N (parts per 100,000)	
		Sample I		
1	0·150	0·010	0·140	93·33
2	0·150	0·008	0·142	94·66
3	0·150	0·015	0·135	90·00
4	0·150	0·026	0·124	82·66
		Sample II		
1	0·0187	0·0090	0·0097	51·87
2	0·0187	0·0072	0·0115	61·50
3	0·0187	0·0120	0·0067	35·83
4	0·0187	0·0110	0·0077	41·18

Experiment 2.

To confirm further the inapplicability of potassium permanganate, neutral or acidic, for the oxidation of small amounts of nitrites present in waters, the following experiment with pure sodium nitrite solutions was carried out.

Treatment.—(1) Ten c. c. of sodium nitrite solution (0·05 mg. N) made up to 50 c. c. and then dilute neutral potassium permanganate added at the laboratory temperature (27—28°C.) till the pink colour persisted and nitrate determined as usual after evaporation to dryness;

(2) Ten c. c. of dilute (sodium nitrite 0·05 mg. N) solution treated with neutral potassium permanganate at 50·C. and nitrate estimated as in (1);

(3) Same as (2) but for the addition of potassium permanganate at 100°C;

(4) Ten c. c. of dilute sodium nitrite (0.05 mg. N) solution treated with acidified potassium permanganate at the laboratory temperature (27—28°C.) and nitrate estimated as in (1);

(5) As in (4) but for the oxidation of the nitrite by acid potassium permanganate at 50°C;

(6) Similar to (4) but for the oxidation of the nitrite at 100°C.

The experiment was duplicated for all treatments and the averagere sults obtained in each case are recorded in Table II.

TABLE II

Nitrite—N added and recovered as nitrate—N under different treatments

Treatment No.	Nitrite—N added (mg.)	Nitrite—N recovered as nitrate—N after oxidation with $KMnO_4$, average of two estimations	Per cent recovery of nitrite—N as nitrate—N
1	0·05	0·0225	45·00
2	,,	0·0247	49·40
3	,,	0·0252	50·04
4	,,	trace	*nil*
5	,,	,,	,,
6	,,	,,	,,

The results show that the recovery of the added nitrogen varies from 45 to 50 per cent where neutral potassium permanganate was employed for oxidation, irrespective of temperature considerations, and practically nothing in cases where acidified potassium permanganate was used.

Experiment 3

As an alternative to the potassium permanganate oxidation, hydrogen peroxide (Merck's) containing twelve volumes of oxygen was tried with varying quantities of sodium nitrite (A. R.) solution, 1 c. c. of which is equivalent to 0·005 mg. N before proceeding to sewage proper. The nitrite solutions were diluted to 50 c. c. each and treated with 5 c. c. of hydrogen peroxide added in small quantities of 0·5 c. c. followed by repeated boiling. A control was also run with hydrogen peroxide to make allowance for any nitrate that might be present in it. The results recorded in Table III show that practically all the added nitrites have been recovered. The hydrogen peroxide was tested and found free from nitrate.

TABLE III

Nitrite—N added and the nitrate—N recovered after oxidation with H_2O_2

No. of dilutions	Nitrite—N added (mg.)	Nitrate—N after oxidation with H_2O_2, average of two estimations (mg.)	Per cent recovery of the nitrite—N as nitrate—N after oxidation with H_2O_2
1	0·025	0·0246	98·4
2	0·040	0·0398	99·5
3	0·050	0·0493	98·6
4	0·075	0·0752	100·27

Experiment 4

The method was next extended to sewage proper adopting the following treatments and the results compared with those obtained by Harper's [1924] method.

Treatment.—(1) Oxidation of the clarified effluent after neutralisation and removal of chlorides, with successive additions of hydrogen peroxide in small lots of 0·5 c. c. each (the total quantity of hydrogen peroxide may vary from 2 to 5 c. c.) followed by repeated boiling ;

(2) Same as (1) without clarification ;

(3) Determination of nitrates in the sample according to Harper's [1924] method.

Blanks were run with a known quantity of hydrogen peroxide and nitrite in each case determined in a separate aliquot of the filtrate. The results recorded in Table IV for the total nitrates after oxidation are quite promising, being at any rate, unlike in the potassium permanganate method, greater than the actual nitrites in the sample. The free nitrates estimated by Harper's [1924] method in the original sample were, however, far below those obtained by the oxidation method even after making allowance for the nitrite in the sample.

TABLE IV

Nitrate—N in the effluent, obtained by the H_2O_2 oxidation process and Harper's method

Treatment	Nitrate—N, average of 4 estimations (parts per 100,000)	Nitrate—N total, after oxidation with H_2O_2, average of 4 determinations (parts per 100,000)	Free nitrate—N in the sample by difference (parts per 100,000)
1	0·038	0·252	0·214
2	0·038	0·253	0·215
3 Direct determination of Nitrate—N by Harper's method .	..	..	0·123

Experiment 5

In this experiment a known amount of nitrate (0·05 mg. of nitrite—N) was added to a definite volume of effluent from the Sludge plant after clarification and removal of chlorides. The recovery of the nitrite nitrogen added was effected almost completely as nitrate nitrogen after oxidation with hydrogen peroxide free from nitrate. A control was also run with the effluent under identical conditions to make allowance for the nitrite and nitrate nitrogen present therein. As the sample in question was of very low nitrite content, less than one part per million, nitrates were also estimated directly both in the control and the sample to which known amounts of nitrite were added according to the standard method [1925] without preliminary oxidation. From the results of the experiment recorded in Table V, it is clear that the nitrate nitrogen obtained without oxidation is very much below that of the oxidised sample, even after deducting the little nitrite nitrogen present therein. It would appear therefore from this experiment that the oxidation of the clarified effluents by hydrogen peroxide is quite essential even when the nitrites present therein are very low.

TABLE V

Nitrate recovery in effluent by the oxidation and direct processes

Sample	Nitrate—N added (mg.)	Nitrite—N in the sample (mg.)	Total N after oxidation, average of four estimations (mg.)	Nitrate—N oxidation method (mg.)	Nitrate—N by the direct method, average of four estimations (mg.)	Per cent loss of nitrate—N, direct method over the oxidation procedure
Effluent alone . . .	*Nil*	0·0076	0·0325	0·0249	0·0125	—50·20
Effluent+sodium nitrate .	0·05	0·0076	0·0820	0·0744	0·0293	—39·39
Recovery of added N by the direct and oxidation methods by difference .	...	...	...	0·0495	0·0168	...

The following procedure may be finally recommended for estimation of nitrates in polluted and other waters.

One hundred to two hundred c. c. of effluent or water are treated with enough freshly precipitated aluminium hydroxide (10 c. c. for every 100 c. c. will be sufficient) followed by the addition of a calculated quantity of silver sulphate solution (8 grms. per litre) to remove the chlorides present in the sample. The mixture is stirred well and filtered. A convenient aliquot from the filtrate corresponding to 50—100 c. c. of the original sample, according to its nitrate content, is neutralised very nearly with dilute sulphuric acid using methyl orange as indicator and then treated with nitrate free hydrogen peroxide (about 3 —5 c. c. for every 50 c. c. have been found sufficient) in small quantities of 0·5 c. c. each followed by repeated boiling for a few minutes. The sample after oxidation is evaporated to dryness in a porcelain dish on a water bath and the residue treated and stirred well with 2 c. c. of Phenol-disulphonic acid. After allowing the mixture to stand for ten minutes it is taken up with 15—20 c. c. of distilled water and transferred to a Nessler cylinder. Then 1 : 1 ammonia is added till the yellow colour appears and the final volume made up to the 100 c. c. mark, after the mixture has cooled down to the laboratory temperature. The liquid is stirred well and the nitrate nitrogen determined by comparison with freshly prepared nitrate standards.

DISCUSSION

The use of acid potassium permanganate for oxidation of nitrate solutions is common and under greatly dilute conditions of this reaction oxidation takes place quantitatively. But the utter disappointment met with when this reagent was used for the determination of small quantities of nitrates present in waters and effluents by the phenoldisulphonic acid method may be due to the following causes :—

(1) It is a recognised fact that when a nitrite solution is treated with standard potassium permanganate in acid medium, it is necessary to use a very dilute solution containing at least 5,000 parts of water for one of nitric

acid to avoid decomposition of the nitrate by sulphuric acid. When the nitrite solution after oxidation with acid potassium permanganate is evaporated to dryness, the concentration of the residual acid, if any, will increase and decompose the nitrate formed in the beginning. This appears more probable in view of the blank results recorded (Table II) in cases where acid potassium permanganate was resorted to for the oxidation of nitrites.

With neutral potassium permanganate as the oxidising agent, the reasons for the loss of nitrate are much less clear. When hydrogen peroxide is used as an oxidising agent, it has been found that the recovery of added nitrite nitrogen to effluent as nitrate nitrogen was almost complete. It is also evident from the results recorded in Table IV that the nitrate nitrogen obtained without oxidation for a sample of very low nitrite content, less than one part per million, is very much below that of the oxidised sample, even after making allowance for the nitrite nitrogen present therein. This low value might be due to the partial reduction of the nitrates by the soluble organic matter present in the clarified effluent during evaporation to dryness. The observation incidentally gives a clue to the probable role, the soluble colourless organic matter and other reducing substances in a clarified sample would play in the determination of nitrates. It looks as though it is absolutely essential, whether there are appreciable quantities of nitrites or not, to oxidise the reducing substances that may be present in clarified effluents or waters free from chlorides, for correct estimations of nitrates therein.

Summary

For a correct estimation of nitrates in sewage and other polluted waters the preliminary oxidation of nitrites, organic matter and other reducing substances in the clarified samples is imperative and for this purpose hydrogen peroxide has been found more effective and reliable than potassium permanganate.

Details of the technique adopted have been outlined.

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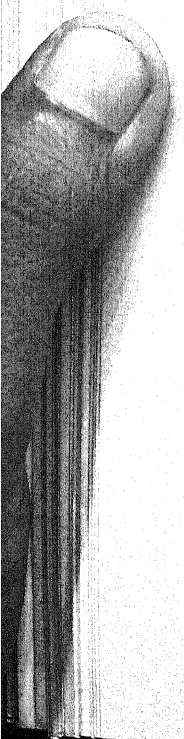

SOME CITRUS NURSERY TECHNIQUE TRIALS AT THE FRUIT RESEARCH STATION, ANANTARAJUPET, MADRAS PRESIDENCY

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(With Plate XXXI)

Introduction

The method of bud-insertion in citrus trees is fairly standardised in this country. It consists of the removal of bud-shield, which carries with it a small portion of the wood of the bud-stick at the time of primary slicing out, but which is carefully removed before insertion of the bud-shield within the " T " or the inverted " T " shaped slit made on the seedling rootstocks. On the other hand, in America [Camp, 1931 ; Coit, 1930 ; Hume, 1926 ; and Wickson, 1926] and in the West Indies [Commission Agriculture, 1905] the general practice at present is to insert the bud-shield with a piece of wood attached to it. Even in the case of other fruits, the American writers advise the retention of a slice of wood in the bud-shield [Bailey, 1903, 1920 ; Chandler, 1928 ; Knapp, 1929 ; and Stahl, 1925]. In South Africa, it has been pointed out that the retention or otherwise of the slice of wood in the bud-shield is largely a method of individual preference or experience [Powell, 1930]. In England, Baltet [1910] has also advocated the retention of a thin slice of wood in the bud-shield, while removal of wood is favoured in some other English publications [Hall and Crane, 1933 and Ministry of Agriculture, 1935]. Work on apples in England has shown that the American practice of retaining the slice of wood in the inserted bud-shield has not established the superiority over the other method, although it has been pointed out that the results are required to be confirmed by further trials during different seasons [Garner, 1934]. The American method has also been reported to have afforded no material benefit in the trials conducted in Palestine. Writers in Australia are divided in their opinion with regard to the advantage or otherwise of retaining a slice of wood [Anon, 1932 and Cock, 1937].

In regard to the optimum period when the seedling rootstocks of citrus are to be lopped after bud-insertion, there is also some divergence of opinion prevailing among citrus nurserymen in most parts of the world. It appears

that in this country, most of the nurserymen lop off the citrus rootstocks either immediately after bud-insertion or within about five to six weeks afterwards or after the bud-sprouts have put forth two to six inches of new growth. Bailey [1903 and 1920] suggests that in outdoor conditions the rootstocks should not be cut back to the bud in one operation but in two ; the first one, soon after the stocks show signs of growth activity when the stock is cut back to a stub over the bud-joint, and second, about two weeks later after the bud has begun to grow, when the stock is cut off to bud-joint. Almost similar recommendations are made by Powell [1930], who further states that, if the stock is cut off close to the bud in the first instance, it might die back and cause the death of the bud. Davies [1917] in South Africa recommends cutting back of the stock to three or four inches above the bud as soon as these are about to shoot out.

The present investigations have been undertaken with a view to ascertain the desirability or otherwise of retention of the slice of wood in the orange bud-shield and the optimum period for carrying out the primary lopping of the rootstocks. The most suitable period for budding oranges in this Station was also intended to be ascertained.

Material and methods

The trial was conducted during the seasons 1936-37 and 1937-38. In the former year, scion was obtained from a single tree of Kodur *chinee* orange, *C. sinensis* Osbeck, and the buds were inserted on eighty seedlings from the same parent of Guntur sour orange, *C. aurantium* Linn. *var.* Hort., every month and during a period of four successive months, commencing from June 1936. One operator was responsible for all the 320 bud-insertions. All the 320 seedlings were growing together in a compact plot of the same age and of apparently uniform vigour. The age of the seedling rootstocks at the time of the first bud-insertion on 9th June 1936 was about twelve months.

The following four treatments were included under study and twenty seedling rootstocks in each of the four months were budded or lopped off according to each of the treatments :—

Treatment No. 1.—Bud inserted with a thin slice of wood adhering to it, and stock seedling lopped off to about three inches above the bud after the bud-sprout has made extension growth of not less than two inches in length.

Treatment No. 2.—Bud insertion after removing the wood from the bud-shield, and stock seedling lopped as in Treatment No. 1.

Treatment No. 3.—Bud inserted as in Treatment No. 1, and stock seedling lopped off to three inches above the bud immediately after bud-insertion.

Treatment No. 4.—Bud inserted as in Treatment No. 2 and stock lopped as in Treatment No. 3.

The second lopping of the rootstocks up to the bud in all the four treatments was done only at the time of lifting the budded plants from nursery beds. In every series, records of the percentage "take" of buds, and the days taken for "bud-break" were collected and recorded. Exactly four months after the date of bud-insertion, the height measurements of bud-sprouts were also collected in every case.

The results obtained during 1936-37 were so striking and interesting that it was considered necessary to lay out a more comprehensive trial in the following year. This consisted of a series of three separate trials with the same treatments as described below :—

(*a*) Seedling rootstocks from one *kichili* (*C. maderaspatana* Tanaka) parent treated by four different methods as explained in 1936 trials, with the scion obtained from a single *chinee* orange (*C. sinensis* Osbeck) parent. The bud-insertions were done during the last week of July 1937 by one operator.

(*b*) Seedling rootstocks from a second *kichili* (*C. maderaspatana* Tanaka) parent treated as above, using scion from a second *chinee* orange (*C. sinensis* Osbeck) parent. The bud-insertions were done from 7th to 11th August 1937 by a second operator.

(*c*) Seedling rootstocks raised from the second *kichili* (*C. maderaspatana* Tanaka) parent referred to under (*b*) above and treated similar to (*a*) and (*b*) using scion from a single acid lime (*C. aurantifolia* Swingle) parent. The buds were inserted from 16th to 20th August 1937 by the same operator mentioned under (*b*).

The seeds of all the rootstocks used in the three aforementioned trials had been sown on the 7th November 1935 and the primary transplantation from seed to nursery beds had been done on the 15th September 1936. All the seeds had been sown on raised seed-beds of made up soil in a small area in a compact plot. The transplantation of the seedlings reserved for each of the three trials had been done to three separate but contiguous plots. Roughly 600 seedlings of apparently uniform size had been planted to nursery beds for each of the three trials.

During the first week of July 1937, *i.e.*, almost immediately before the insertion of orange buds, measurements of stem thickness of individual seedlings growing in the plot reserved for the first of these three trials were collected. The analysis of these measurements showed that the mean of 352 plants was 0·83 cm. with a standard deviation of 0·21, and the coefficient of variability was therefore 25·3 per cent. In order to show a 10 per cent difference between the two means it would require eighty-four observations for a level of $P = 0{\cdot}01$ [Hoblyn, 1931 and Vaidyanathan, 1936]. Neither the area nor the number of plants available rendered the selection of such a large number of individuals possible, having regard to the necessity of providing an adequate number of guard rows. Furthermore, it was expected on the basis of the results of 1936 that the differences between the treatments would be

of fairly large proportions. The fact that measurements collected related to only the seedling rootstocks, whereas the ultimate data are to be collected on scion worked on these rootstocks, renders it also not absolutely necessary to base the number of replications strictly on growth measurements of the root-stocks.

It was, therefore, decided to have four repetitions of rows, containing a minimum number of sixteen buddable seedlings, thus involving in all a minimum of sixty-four buddable seedlings under each treatment in each of the three trials.

The number of treatments randomised in each block was four, and this was identical to the four treatments described above.

The following was the plan of layout of trials designated as (*a*) on page 653.

Guard rows

Guard rows	Block I	Block II	Block III	Block IV	Guard rows
	1	3	3	1	
	2	1	2	3	
	3	4	1	2	
	4	2	4	4	

Guard rows

A similar plan of layout was adopted in the other two trials also, with a separate randomisation of plots in the last case.

The total number of seedlings budded by each of the four treatments in each block in trial (*a*) is given below :—

Blocks	Treatment No. 1	Treatment No. 2	Treatment No. 3	Treatment No. 4	Total
I . . .	16	16	16	16	64
II . . .	16	17	16	16	65
III . . .	19	17	16	19	71
IV . . .	16	17	18	20	71

In order to ascertain the optimum season for budding sweet oranges to seedling rootstocks, a separate investigation was planned out in 1936 independently of the abovementioned trials. Two citrus varieties—*kichili* (*C. maderaspatana* Tanaka) and *gajanimma* (*C. pennivisiculata* Tanaka) which are grown fairly extensively in this tract—were selected for these trials. The scion was selected from a single *chinee* orange tree, and the bud-insertions were made by one operator every month from June 1936 to May 1937. From June to September 1936, the number of rootstocks of each of these varieties budded every month varied from ten to seventeen according to the availability of seedlings in a compact plot and uniformity of vigour. During the remaining months buds were inserted on to twenty seedlings of each of these rootstocks. The age of the rootstocks, however, could not be kept constant during every bud-insertion owing to the practical difficulties involved in securing seeds for sowing every month. The budding technique employed in the experiment was the one described under method No. 1 above.

Results of 1936-37 trials

The data collected during 1936 are summarised and presented in Table I.

TABLE I

"Bud-take", "Bud-break" and growth of the bud-sprouts, four months after bud-insertion

Stock—Guntur sour orange *Scion*—Chinee *orange*

Date of bud insertion	Method No. 1			Method No. 2			Method No. 3			Method No. 4		
	"Take"	"Bud-break" period in days	Height	"Take"	"Bud-break" period in days	Height	"Take"	"Bud-break" period in days	Height	"Take"	"Bud-break" period in days	Height
	Per cent		(cm.)	Per cent		(cm.)	Per cent		(cm.)	Per cent		(cm.)
9-6-1936 . .	67·0	30 to 40	16·4±1·85	42·0	30 to 40	14·5±1·57	56·0	10 to 15	10·6±1·15	25·0	10 to 15	11·5±1·59
7-7-1936 . .	100·0	Do.	16·0±1·67	83·0	Do.	14·0±1·21	75·0	Do.	10·0±2·20	71·0	Do.	8·0±1·14
11-8-1936 . .	83·0	Do.	12·5±1·17	62·0	Do.	9·0±0·59	61·0	Do.	5·7±0·62	50·0	Do.	2·1±0·38
7-9-1936 . .	100·0	Do.	18·4±2·06	65·0	Do.	13·2±0·89	70·0	Do.	11·5±1·93	40·0	Do.	11·7±2·31

The following interesting facts appear to have emerged from this investigation :—

(*i*) The number of plants produced from buds with wood attached to the bud-shield is consistently greater than the number produced from those inserted after removal of wood on identically lopped rootstocks. The difference is so large and the similarity of results under different seasons so strikingly great that, the superiority of inserting the bud with the wood over the common Indian method of inserting the bud after removal of wood may be said to be clearly established.

(*ii*) " Bud-break " appears to be clearly stimulated by lopping of the stocks at the time of bud-insertion. A difference of three weeks is noticeable in favour of lopped stocks in all the four months.

(*iii*) The height measurements of the bud-sprouts collected four months after bud-insertion also reveal consistently significant differences. In spite of an earlier " bud-break " on lopped rootstocks, it will be noticed that the amount of growth in terms of length of new shoots on the lopped stocks is markedly less than on those stocks which were lopped one month after insertion. The data, therefore, indicate that the budded plants will be available for planting within a shorter period of time if the lopping operation is delayed for a month.

(*iv*) The presence of wood in the bud-shield does not appear to be an important factor in determining the growth processes on the rootstocks, as the lopping treatment.

(*v*) Both in the matter of " bud-take " and subsequent growth of bud-sprouts, interesting indications of the seasonal effects have been afforded by these data. July has proved to be the optimum month for budding operations, followed by September, while in June, the " bud-take " has been uniformly poor under all the four treatments.

(*vi*) The rate of growth of bud-sprouts has been comparatively more rapid in the September batch. June and July may also be classed as favourable months in this respect, while August has proved to be responsible for a comparatively poor rate of growth on this rootstock.

It seems safe to infer from these data that July and September are the optimum months for the propagation of *chinee* orange by budding in this tract, and that the presence of a slice of wood in the bud-shield and delayed lopping of the rootstocks till the bud-sprouts have made a sufficient growth of not less than two inches in length, are conducive for producing not only a large number of plants but also an earlier maturation of bud-sprouts.

Results of the trial on 'the optimum season for budding' carried out during 1936-37

The results of the separate investigation carried out to ascertain the optimum season for budding of oranges are presented in Table II.

TABLE II

Percentage "bud-take" of chinee *orange on the rootstock varieties, 1936-37*

Months	Stocks		Mean
	Kichili	*Gajanimma*	
June	77	90	83·5
July	100	100	100·0
August	88	82	85·0
September	95	100	97·5
October	55	60	57·5
November	20	60	40·0
December	45	75	60·0
January	90	90	90·0
February	90	90	90·0
March	20	25	22·5
April	45	50	47·5
May	30	45	37·5
Mean	62·9	72·3	

There is a fairly wide difference in the percentage "take" of orange buds between the two rootstock varieties in favour of *gajanimma*, which produces about 9 per cent more "bud-take" in the case of the twelve months, while the variations due to several months are also highly marked.

On the basis of the above results, the months are arranged below in the descending order of magnitude in percentage "take":—July, September, January, February, August, June, December, October, April, November, May, March.

The optimum months for successful budding were, therefore, July and September. During the remaining months high "take" of buds were obtained during January, February, August and June while April, November, May and March were found to be decidedly the most unfavourable.

July and September, therefore, have proved to be the most favourable months for budding oranges on three rootstock varieties in two independent trials.

From a reference to the rainfall and temperature records during 1936-37, it appears that the months which have proved to be the most unfavourable for budding operations have been such that have either been characterised by heavy precipitations or prolonged hot and dry spells. For instance, the heaviest rainfall recorded during the year was in November, while the maximum temperature under shade had reached above 100°F. only during March, April and May. It appears possible, therefore, that extremes in wet weather or temperature are both important limiting factors in the propagation of citrus by means of budding.

It has also been observed that excessive dry and hot weather prevailing between March to June has considerably injured the young sprouts. A few sprouts emerging from the February insertions also were damaged by the hot weather prevailing in the following months.

The suitability of February and possibly of the latter half of January for the operation is, therefore, open to question in spite of the high percentage of success obtained during these months. Considering all these points, it seems that July to September provides the optimum period for budding oranges in this tract, but the suitability of any of these months may be influenced by the wet weather conditions prevailing during the bud-insertion period. The latter point seems to be of importance, in that, while the month of August produced less success during 1936 than July and September, during 1937 it was found that August produced the maximum percentage of success in all the series of bud-insertions totalling about 2,000 on about six different rootstocks, while July to September, though continued to produce high "take", were much behind August in this matter.

Results of 1937-38 trials

Trial (a)

All percentages have been analysed after the Sin^{-1} transformation and 'No. of days' after the square root transformation [Cochran, 1938]. For splitting into single D. F., Yate's [1934] approximate method has been used where there were one or two 'missing' values in some classes.

"*Bud-take*".—The mean percentage "take" and the analysis of variance worked out for the percentage "take" obtained in the 1937 trials are presented in Tables III and IV.

TABLE III

Mean percentages "take" (X)—Nursery Technique Trials

Stock—Kichili. *Scion*—Chinee *orange*.

Serial No.	Blocks	Treatment No. 1	Treatment No. 2	Treatment No. 3	Treatment No. 4	Total	Mean
1	I	68·75	56·25	93·75	56·25	275·00	68·75
2	II	88·24	75·00	87·50	68·75	319·49	79·87
3	III	94·74	76·47	93·75	31·58	296·54	74·14
4	IV	93· 5	52·94	94·44	37·50	278·63	69·66
5	Total	345·48	260·66	369·44	194·08	1169·66	..
6	Mean	86·37	65·17	92·36	48·52	Gen. Mean :	73·10

TABLE IV

Analysis of variance of Sin$^{-1}\sqrt{x}$

Due to	D. F.	Sum of squares	Mean square	Remarks
Blocks	3	102·91	34·30	Not significant
Wood *vs.* no wood . .	1	2072·52	2072·52	Highly significant
Lopping *vs.* no lopping .	1	28·35	28·35	Not significant
Interaction . . .	1	211·99	211·99	Not significant
Treatments . . .	3	2312·86	770·95	Highly significant
Error	9	668·66	74·29	
Total	15	3084·43		

It is clear that insertion of buds with wood on has produced a larger number of budlings than insertion of the buds after the removal of wood, the average increase percentage being 32·5. This result is in conformity with that obtained during 1936.

Lopping treatment has not affected the percentage "take".

"*Bud-break*".—The period taken for "bud-break" was collected in every case as in the previous year and results are summarised in Tables V and VI.

TABLE V

Mean number of days (x) *taken for* "*Bud-break*"

Stock—Kichili *Scion*—Chinee *orange*

Serial No.	Blocks	Treatment No. 1	Treatment No. 2	Treatment No. 3	Treatment No. 4	Total	Mean
1	I	16·00	16·00	9·00	12·00	53·00	13·25
2	II	18·00	17·00	11·00	11·00	57·00	14·25
3	III	13·00	17·00	11·00	12·00	53·00	13·25
4	IV	16·00	16·00	9·00	12·00	53·00	13·25
5	Total	63·00	66·00	40·00	47·00	216·00	..
6	Mean	15·75	16·50	10·00	11·75	Gen. Mean :	13·50

TABLE VI

Analysis of variance of $\sqrt{x}$

Due to	Degrees of freedom	Sum of squares	Mean square	Remarks
Blocks	3	0·0507	0·0169	
Wood *vs.* no wood . .	1	0·1462	0·1462	Not significant.
Lopping *vs.* no lopping .	1	2·0686	2·0686	Highly significant.
Interaction . . .	1	0·0185	0·0185	Not significant.
Treatments . . .	3	2·2333	0·7444	Highly significant.
Error	9	0·3151	0·0350	..
Total	15	2·5991	..	..

Lopping has clearly stimulated an earlier "bud-break". On the average, lopping has effected an earliness of 5·25 days in "bud-break" and presence of wood by 1·25 days.

Maturation of bud-sprouts.—In February 1938, the total extension growths made by the bud-sprouts were collected in the case of all budlings, and these are summarised in Tables VII and VIII.

TABLE VII

Mean extension growth in cm. Nursery Technique Trials

Stock—Kichili *Scion*—Chinee *orange*

Serial No.	Blocks	Treatment No. 1	Treatment No. 2	Treatment No. 3	Treatment No. 4	Total	Mean
1	I	32·60	47·10	40·00	23·67	143·37	35·84
2	II	51·83	25·55	45·13	29·67	152·18	38·05
3	III	61·56	44·50	34·44	48·50	189·00	47·25
4	IV	50·78	55·00	32·54	30·66	168·98	42·25
5	Total	196·77	172·15	152·11	132·50	653·53	..
6	Mean	49·19	43·04	38·03	33·13	Gen. Mean	40·85

TABLE VIII

Analysis of variance for mean extension growth

Due to	Degrees of freedom	Sum of squares	Mean square	Remarks
Blocks	3	303·44	101·15	Not significant.
Wood *vs.* no wood . .	1	121·76	121·76	Not significant.
Lopping *vs.* no lopping .	1	444·32	444·32	Not significant.
Interaction . . .	1	1·44	1·44	Not significant.
Treatments . . .	3	567·52	189·17	Not significant.
Error	9	1044·50	116·06	..
Total	15	1915·46	..	..

It is evident that, although earlier lopping produces a slightly less growth of bud-sprouts than the plants lopped at a later stage, the differential effects cannot be termed as significantly greater in favour of any one treatment.

Trial (b)

The summary of the results and analysis of variance of the second trial are presented in Tables IX and X.

TABLE IX

Percentage "take" (x)

Stock—Kichili, *2nd parent* *Scion*—Chinee *orange—2nd parent*

Serial No.	Blocks	Treatment No. 1	Treatment No. 2	Treatment No. 3	Treatment No. 4	Total	Mean
1	I	75·00	58·82	56·25	50·00	240·07	60·02
2	II	87·50	64·28	56·25	18·75	226·78	56·70
3	III	93·75	33·33	88·24	31·25	246·57	61·64
4	IV	81·25	64·23	94·12	[00·00]	239·65	59·91
5	Total	337·50	220·71	294·86	100·00	953·07	..
6	Mean	84·38	55·18	73·72	25·00	Gen. Mean:	59·56

TABLE X

Analysis of variance of $Sin^{-1}\sqrt{x}$

Due to	Degrees of freedom	Sum of squares	Mean square	Remarks
Blocks	3	462·94	154·31	Not significant.
Wood *vs.* no wood . .	1	1858·37	1858·37	Highly significant.
Lopping *vs.* no lopping .	1	377·81	377·81	Not signifi an .
Interaction . . .	1	40·33	40·33	Not significant.
Treatments . . .	3	2111·91*	703·97	Highly significant.
Error	8	718·36	89·79	..
Total	14	3293·21	..	..

* Separately estimated and not the total of three single degrees of freedom [Yates, 1934].

The above data show that bud-shield with wood has again contributed to a significantly larger percentage " bud-take " than bud-shield without wood, the average increase in favour of the former being 38·96 per cent. Lopping has again had no effect on the percentage " take ".

TABLE XI

Mean number of days (x) *for " bud-break "*

Stock—Kichili, *2nd parent* *Scion*—Chinee *orange—2nd parent*

Serial No.	Blocks	Treatment No. 1	Treatment No. 2	Treatment No. 3	Treatment No. 4	Total	Mean
1	I	13·00	16·00	7·00	8·00	44·00	11·00
2	II	14·00	16·00	10·00	7·00	47·00	11·7
3	III	12·00	13·00	7·00	14·00	46·00	11·50
4	IV	14·00	14·00	9·00	..	37·00	9·25
5	Total	53·00	59·00	33·00	29·00	174·00	..
6	Mean	13·25	14·75	8·25	7·25	Gen. Mean :	10·88

TABLE XII

Analysis of variance of $\sqrt{x}$

Due to	Degrees of freedom	Sum of squares	Mean square	Remarks
Blocks	3	0·0878	0·0293	Not significant.
Wood *vs.* no wood . .	1	0·1526	0·1526	Not significant.
Lopping *vs.* no lopping .	1	2·1959	2·1959	Highly significant.
Interaction . . .	1	0·0001	..	Not si nificant.
Treatments . . .	3	2·4530*	0·8177	Significant.
Error	8	1·0181	0·1273	..
Total	14	3·5589	..	..

* Separately estimated and not the total of the three single degrees of freedom [Yates, 1934].

It is evident that lopping has distinctly stimulated an earlier break of buds, the average difference between lopped and unlopped plants being 6·25.

Presence or absence of wood has not produced any significant difference in respect of "bud-break", in the case of plants lopped in a similar manner.

TABLE XIII

Mean extension growth (in cm.)

Stock—Kichili, *2nd parent* *Scion*—Chinee orange—*2nd parent*

Serial No.	Blocks	Treatment No. 1	Treatment No. 2	Treatment No. 3	Treatment No. 4	Total	Mean
1	I	46·14	52·50	21·70	6·00	126·34	31·59
2	II	37·50	49·25	20·66	7·00	114·41	28·60
3	III	41·92	29·33	23·33	52·50	147·08	36·77
4	IV	34·44	47·00	19·25	..	100·69	27·67
5	Total	160·00	178·08	84·94	65·50	88·52	..
6	Mean	40·00	44·52	21·24	16·38	Gen. Mean :	30·53

TABLE XIV

Analysis of variance

Due to	Degrees of freedom	Sum of squares	Mean square	Remarks
Blocks	3	207·76	69·25	Not significant.
Wood *vs.* no wood . .	1	0·12	0·12	Not significant.
Lopping *vs.* no lopping .	1	2199·52	2199·52	Significant.
Interaction . . .	1	88·04	88·04	Not significant.
Treatments . . .	3	2287·68	762·56	Not significant.
Error	9	1875·21	208·36	..
Total	15	4370·65	..	..

A distinctly larger extension growth is observed in plants which are lopped after the bud-sprouts have produced some growth, the average increase in extension growth in favour of unlopped plants being 23·45 cms., and the critical difference 17·07. Presence or absence of wood has not significantly affected the extension growth.

In general, it is possible to state on the basis of these two trials that :—

(*i*) the presence of wood in the orange bud-shield has contributed to over 32 per cent higher " take " of buds on *kichili* rootstocks ;

(*ii*) lopping of *kichili* rootstocks at the time of bud-insertion has not affected the percentage " take " of orange buds ;

(*iii*) lopping of *kichili* rootstocks has stimulated an earlier "bud-break" by over five days in *chinee* orange ; and

(*iv*) presence or absence of wood in the *chinee* orange bud-shield has shown to exert no material influence on the extension growth of bud-sprouts on *kichili*.

Trial (c)

The data on " bud-take " of acid lime scion parent are presented in Tables XV and XVI.

TABLE XV

Percentage " take " (x)

Stock—Kichili, *2nd parent* *Scion—Acid lime*

Serial No.	Blocks	Treatment No. 1	Treatment No. 2	Treatment No. 3	Treatment No. 4	Total	Mean
1	I	64·29	23·08	30·00	0·00	117·37	29·34
2	II	75·00	14·28	50·00	33·33	172·62	43·15
3	III	76·47	12·50	41·18	16·67	146·82	36·71
4	IV	73·33	33·33	25·00	10·00	141·66	35·42
5	Total	289·09	83·19	146·18	60·00	578·46	..
6	Mean	72·27	20·80	36·55	15·00	Gen. Mean :	36·15

TABLE XVI

Analysis of variance of $\sin^{-1}\sqrt{x}$

Due to	Degrees of freedom	Sum of squares	Mean square	Remarks
Blocks	3	58·98	19·66	Not significant.
Wood *vs.* no wood . .	1	1682·22	1682·22	Highly significant.
Lopping *vs.* no lopping .	1	452·22	452·22	Significant.
Interaction . . .	1	389·11	389·11	Significant.
Treatments . . .	3	2596·40*	865·46	Highly significant.
Error	8	394·47	49·31	..
Total	14	3049·85	..	..

* Not the total of the three single D. F. but separately estimated [Yates, 1934].

In this scion, the unlopped stocks worked with wood attached to the bud-shield has produced an outstandingly larger "take" of buds than in any other treatment, the increase over any other treatment being 35·72 per cent or over.

Presence of wood has contributed to 36·51 per cent higher "take" of buds than absence of wood, while unlopped plants have produced 20·76 per cent higher "take" than the lopped ones.

TABLE XVII

Mean number of days (x) *for "bud-break"*

Stock—Kichili, *2nd parent* *Scion—Acid lime*

Serial No.	Blocks	Treatment No. 1	Treatment No. 2	Treatment No. 3	Treatment No. 4	Total	Mean
1	I	17·00	31·00	11·00	..	59·00	14·75
2	II	20·00	24·00	15·00	15·00	74·00	18·50
3	III	20·00	29·00	15·00	14·00	78·00	19·50
4	IV	18·00	28·00	16·00	..	62·00	15·50
5	Total	75·00	112·00	57·00	29·00	273·00	..
6	Mean	18·75	28·00	14·25	7·25	Gen. Mean :	17·06

TABLE XVIII

Analysis of variance of $\sqrt{x}$

Due to	Degrees of freedom	Sum of squares	Mean square	Remarks
Blocks	3	0·0965	0·0322	Not significant
Wood *vs.* no wood . .	1	0·8032	0·8032	Not significant
Lopping *vs.* no lopping .	1	3·3594	3·3594	Highly significant
Interaction . . .	1	0·6781	0·6781	Significant
Treatments . . .	3	5·4669*	1·8189	Highly significant
Error	7	0·5220	0·0746	..
Total	13	6·0754	..	..

* Separately estimated and not the total of the three single D. F. [Yates, 1934].

As in the case of orange scion, lopping of rootstocks has stimulated an earlier " bud-break " by an average of 12·63 days than in the unlopped series. Presence or absence of wood does not appear to be a factor which influences the " bud-break " period. Since the number of successfully budded plants under treatment No. 4 is very small and nil in two plots, these large differences may not present a very accurate picture of the effects of lopping on " bud-break ".

As a large number of acid lime bud-sprouts failed to make a good growth and, further, as no bud-sprouts were available on two plots, it was not possible to analyse the results relating to the extension growth made by the plants under this experiment. The following inferences which are drawn from this experiment are, therefore, confined to only the data collected on " bud-take " and " bud-break ".

(*i*) Presence of wood in acid lime bud-shield has contributed to over 36 per cent higher " take " of buds on *kichili* rootstocks.

(*ii*) Lopping of *kichili* rootstocks at the time of bud-insertion has lowered the " take " of acid lime buds by about 20 per cent.

(*iii*) Presence or absence of wood in acid lime bud-shield has not had any effect on the period taken by buds to " break " on *kichili* rootstocks.

(*iv*) Lopping of *kichili* rootstocks has stimulated an earlier " bud-break " in acid limes by an average of over twelve days.

Discussion

Taking the results of all the three trials carried out during 1937-38 into consideration, it is observed in general that the results confirm those obtained in the preceding year, in so far as the beneficial effects of the presence of wood in the bud-shield is concerned. The practice of lopping at the time of bud-insertion has also shown both in the 1936-37 as well as in 1937-38 trials to have a distinctly stimulating effect on the "bud-break". With regard to the extension growth of bud-sprouts as effected by lopping treatment, the results of the two years and also those between the different series in the 1937-38 trials are contradictory to a certain extent. However, when it is remembered that the delayed lopping of rootstocks in all the four months in the 1936-37 trials and in the second trial with *chinee* oranges in 1937-38 have produced significant increases in the extension growths of bud-sprouts over the method of lopping the rootstocks at the time of bud-insertion, and also the extension growth of the bud-sprouts in the first trial in 1937-38 has been higher though not statistically significant in the unlopped series than in the lopped, it appears reasonable to assume that delayed lopping is conducive to an early maturation of bud-sprouts.

Mendel [1937] has shown that the lopping of the rootstocks results in much more serious injury to the plant than the wound caused by the 'T' cut, because of the interference with the supply of the photosynthetic products from the side of the stub. Delayed lopping would therefore appear to be a practice more in consonance with the plant requirements at this stage than lopping at the time of, or immediately after, bud-insertion.

Considering that the removal of wood from bud-shield or the removal of the shield without the wood attached to it requires time and skill and that the removal of the wood involves some risk of injury to the bud, it is suggested that the replacement of the prevalent Indian method of removal of wood from the bud-shield by the American method of retaining the wood will be of considerable practical and economic importance in citrus nursery practices.

Both during 1936-37 and 1937-38, the trials carried out definitely establish the fact that July, August and September provide the optimum period for budding oranges in this tract. The data also seem to reveal that, for securing the most favourable results, budding should be carried out during this period when dry weather prevails. October to January provides the second best period for budding operation, provided the north-east monsoon does not cause heavy precipitations during the budding period and the hot and dry weather does not follow immediately after bud-insertion, as it usually happens in the case of January bud-insertions. If the hot season is not prolonged up to the end of June, a fairly high "take" of buds can be reasonably expected in that month also.

The above results are not in accord with the experiences of the citrus nurserymen in most parts of India. In the matter of the influence of presence of wood in the bud-shield, the results obtained in England [Davies, 1917]

are also contradictory to those obtained in these trials. Garner [1934] has, however, recognised the possibility of seasonal conditions affecting the results and has stated that it has been found necessary in England to insert apple and plum buds with wood attached, because of the conditions of the scion variety, which prevented the removal of wood without injury to the bud. He also points out that the difference in " bud-take " obtained in his trials is very small and unlikely to be of economic importance. In fact, he considers that insertion of bud-shield with wood will lead to economy of time, reduce loss and furnish a more useful method to the amateur budder of apples, pears, plums and cherries.

It is possible that the results may be affected to a considerable extent by the age of the rootstocks, nature of scion wood, skill of operators, period of exposure of the bud-shield prior to insertion and also the nature of the material or solution in which the bud-wood and the separated bud-shield is retained till the time of actual insertion. It may be pointed out that the slice of wood when retained in the bud-shield probably serves a useful purpose in retaining moisture in the bud for a longer period than when without it. Opinion is also likely to be expressed that the wood if retained leads to the interposing of a foreign body between the healing the surfaces, thus possibly preventing the bark of the bud from uniting directly with the exposed surface of the rootstock. Bailey [1920] holds that the youngest portions of the wood in the bud probably unite with the stock, but such union may not be possible when knife cuts are deep and denser part of wood or pith of the trees are retained in the bud-shield. Microscopic examination of the eighty-three transverse sections of the bud-joint of the plants budded with wood on in these trials after about five months of the insertion has not shown the presence of any interposing body between the cambium layers. It, therefore, appears clear that when citrus rootstocks of about twenty months age are budded with bud-shields having a slice of wood, no impediment occurs in the complete union of the two cambium layers.

It has, however, been found that, where buds with very thick slices of wood are inserted, the bud-joint becomes abnormally raised out, forming a gall-like structure. When such galls are very large, the plants die a premature death(Plate XXXI, fig. 2). The size of the wood retained in the bud-shield in the present trial was very small, with a smooth and flat cut surface, not exceeding 2·50 cm.$\times$0·40 cm.$\times$0·15 cm. The size was the same as was obtained by the operator at the time of slicing out from the bud-stick. The operator was prevented from inserting any bud-shield which was not found to the mark or in which case the size of the attached wood had to be reduced after slicing out from the bud-shield. These show that the operator has to be trained sufficiently in order to be able to skilfully slice out the bud-shield of the required kind. Lack of skill on the part of operator, unsuitability of the season and unfavourable treatments given to the bud-shield prior to insertion are, therefore, likely to be a few of the more potent causes for the divergent experience of the citrus nurserymen with regard to the relative merit of bud-insertions with and without wood attached to the bud-shield.

FIG. 2

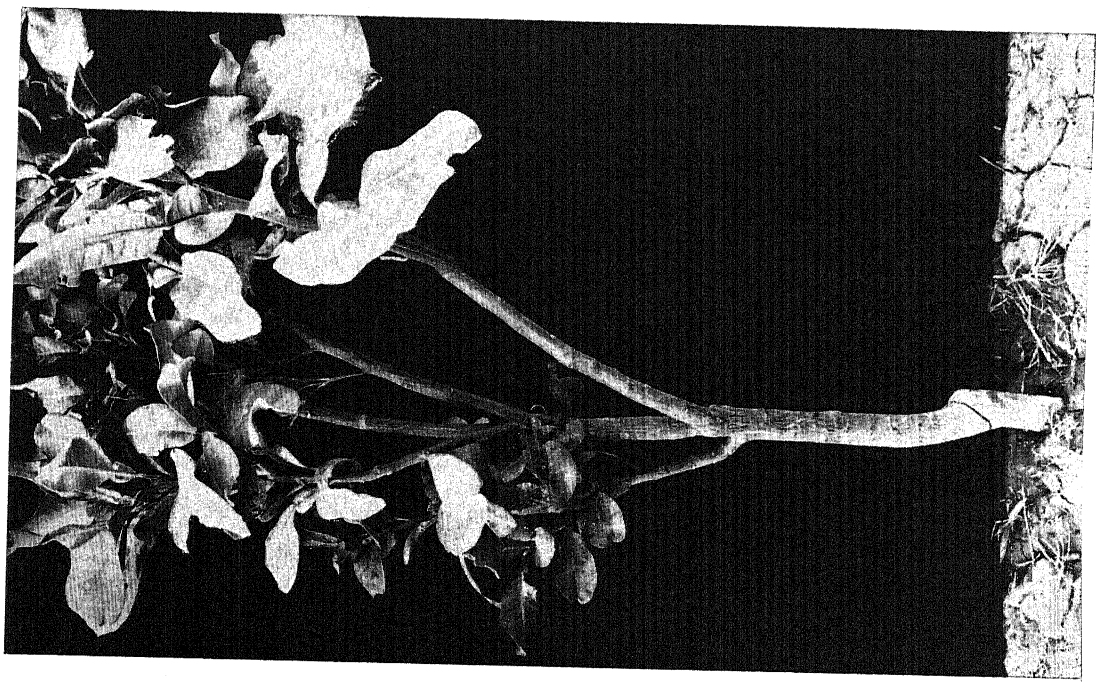
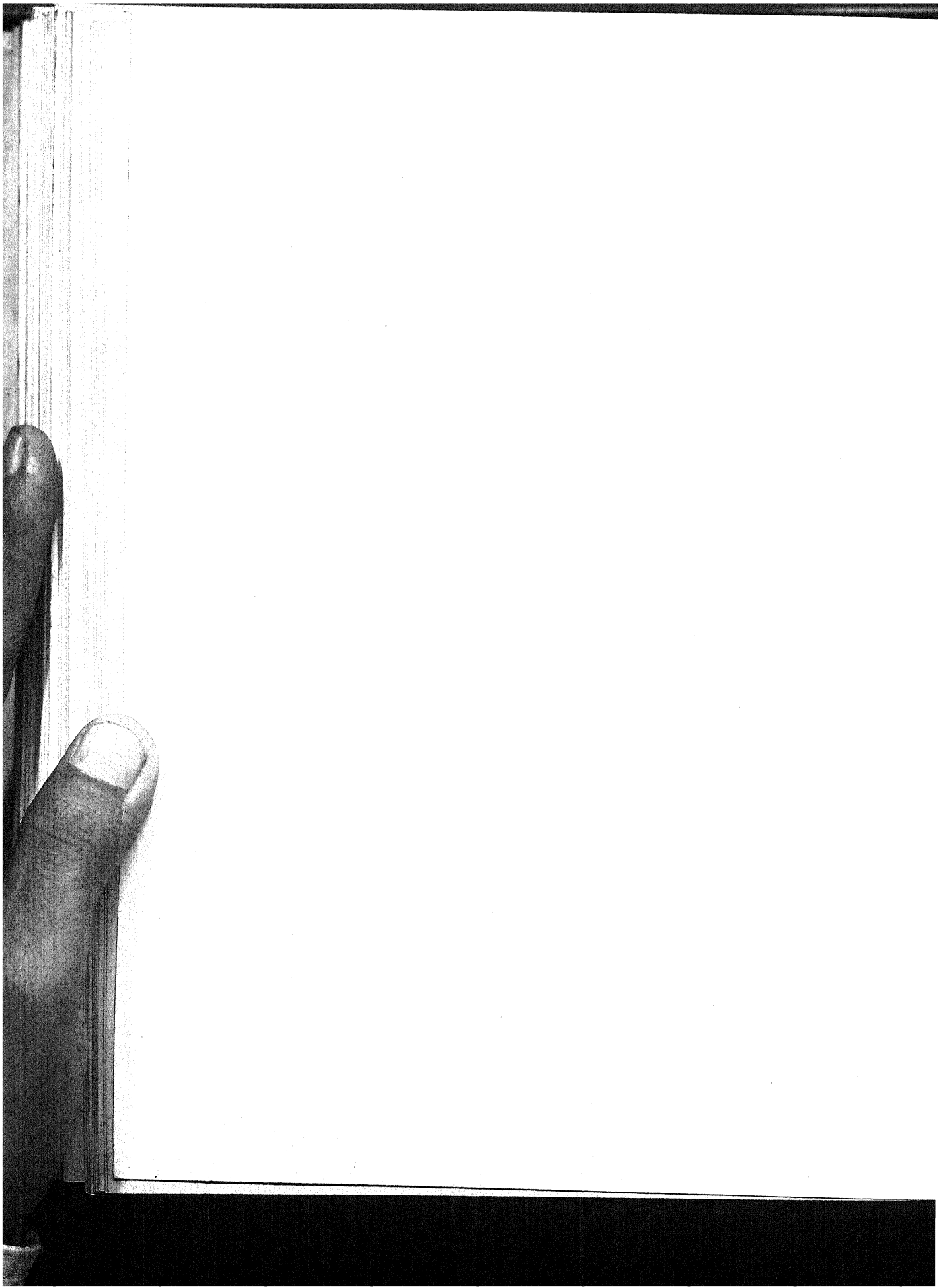


FIG. 1



The formation of a callus pad at the lower end of the inverted 'T' cut caused by exuberant growth has been referred to by Mendel [1937] in his investigations on anatomy and physiology of citrus budding. In properly budded plants, the callus beneath the bud-shield has been shown to cease its growth as soon as the gap between the rootstock and the shield is filled and the resulting pressure renders further cell division impossible. The callus may, however, grow into the cleft formed at the horizontal cut on the rootstock without hindrance from the tissues lying above, thus forming a pad. It is possible that when a rough and ununiform slice of wood is retained in the bud-shield or when the horizontal cut is too large or the bud-shield is ill-fitting, the callus pad may assume large size. If a connecting cambium differentiates in such a pad, as it is reported to do two to three weeks after budding [Mendel, 1937], the activity of this cambium may lead to the formation of more cell layers resulting in the raising out of the bud-shield and formation of a swollen bud-joint instead of a smooth one as shown in Plate XXXI, fig. 1.

That the common practice of the budders to retain the bud-shield in water or inside the mouth of the operator in contact with saliva hinders successful " take " of such buds has been a widely accepted belief. In the experiments reported here, all the bud-shields were separated out immediately before insertion and inserted with the minimum of exposure and without contact to any kind of liquid or storage in any other material or container.

Investigations on the anatomy and histology of citrus budding in Palestine [Mendel, 1937] has shown that, while the outer layers of wood are able to form callus easily, the wounds which penetrate deeper into the wood are closed by only one overgrowth of the callus tissue originating from the peripheral regions of the wounds. This shows that in the hands of an inexperienced budder, the extent of activity of the healing tissue and consequently the success of the bud-union will depend upon the regenerative power of the peripheral regions of the inflicted wounds. It has also been shown in the above-mentioned investigation that the layers between the primary bark and the pith in young citrus rootstocks are not sharply defined, so that when the bark is lifted for budding, it is never separated from the stem exactly at the inner border of the cambium. It would also appear that in the process of making the 'T' or inverted 'T' cut on the rootstock, the cut may reach the interior sapwood still in the initial lignified state, which has not the power to regenerate. Insertion of the bud with a thick slice of wood adhering to it or a careless incision are, therefore, points that may make all the difference between the success and failure of the insertion.

The data set forth in the preceding pages regarding the optimum season for orange budding on *kichili* and *gajanimma* has shown that the latter variety of rootstock produces a higher " take " of buds than the former. The wide difference existing between the various citrus varieties regarding the efficiency of "bud-take " has also been proved in a number of other trials which have not been reported in these pages. These facts go to show that the practical interests of a citrus nurseryman and the grower are not necessarily

identical. While the former may raise oranges on a rootstock which produces the maximum " bud-take ", the latter may find that the variety favoured by the nurserymen may not give the best performance. In fact it has been found at this station that, of all the citrus varieties used as rootstocks, *gajanimma* is the most susceptible to gummosis, while *kichili* appears to be among those that are fairly immune to this disease. In order to select the best rootstock from the viewpoint of the grower and nurseryman elaborate rootstock trials are, therefore, necessary to supplement the results from the nursery technique trials.

Summary

1. Presence of wood in *chinee* orange and acid lime bud-shield has shown to produce a significantly higher " take " of buds on different rootstock varieties than absence of wood.

2. Primary lopping of citrus rootstocks at the time of bud-insertion has lowered the " take " of buds in some cases.

3. Primary lopping of the citrus rootstocks at the time of bud-insertion has stimulated an earlier " bud-break " both in *chinee* oranges and acid limes.

4. Delayed primary lopping of citrus rootstocks, after the inserted *chinee* orange buds have produced not less than two inches of extension growth, has resulted in a comparatively rapid extension growth of bud-sprouts.

5. Presence or absence of wood in the *chinee* orange and acid lime bud-shields has not affected the period taken for " bud-break " or for rapid extension growth of *chinee* orange bud-sprouts.

6. July to September provide the optimum season for budding *chinee* oranges in this tract, while October to January provide the next best period, provided the bud-insertions are done sufficiently before the advent of the hot-weather period. Heavy precipitation during bud-insertions appear to inhibit the " take " of buds.

7. *Gajanimma* produces a significantly higher " take " of buds than *kichili*. This, however, cannot be taken to mean that the former is a more suitable rootstock than the latter.

Conclusion

For producing a larger number of budded plants of *chinee* oranges and acid limes and for encouraging an early maturation of bud-sprouts, insertion of bud-shield with a piece of wood attached to it and primary lopping of the rootstocks after the bud-sprouts have produced not less than two inches of extension growth, have been found to be the most suitable practices.

July to September has proved to be the optimum period for budding *chinee* oranges under the conditions prevailing in this tract. Poor " take " has been shown to result when bud-insertions are made under wet-weather conditions or during hot and dry spells. Hot season, if it follows bud-insertion very soon, has been shown to cause injury to bud-sprouts and retards their subsequent growth.

A higher " take " of orange buds is obtained on *gajanimma* than on *kichili*.

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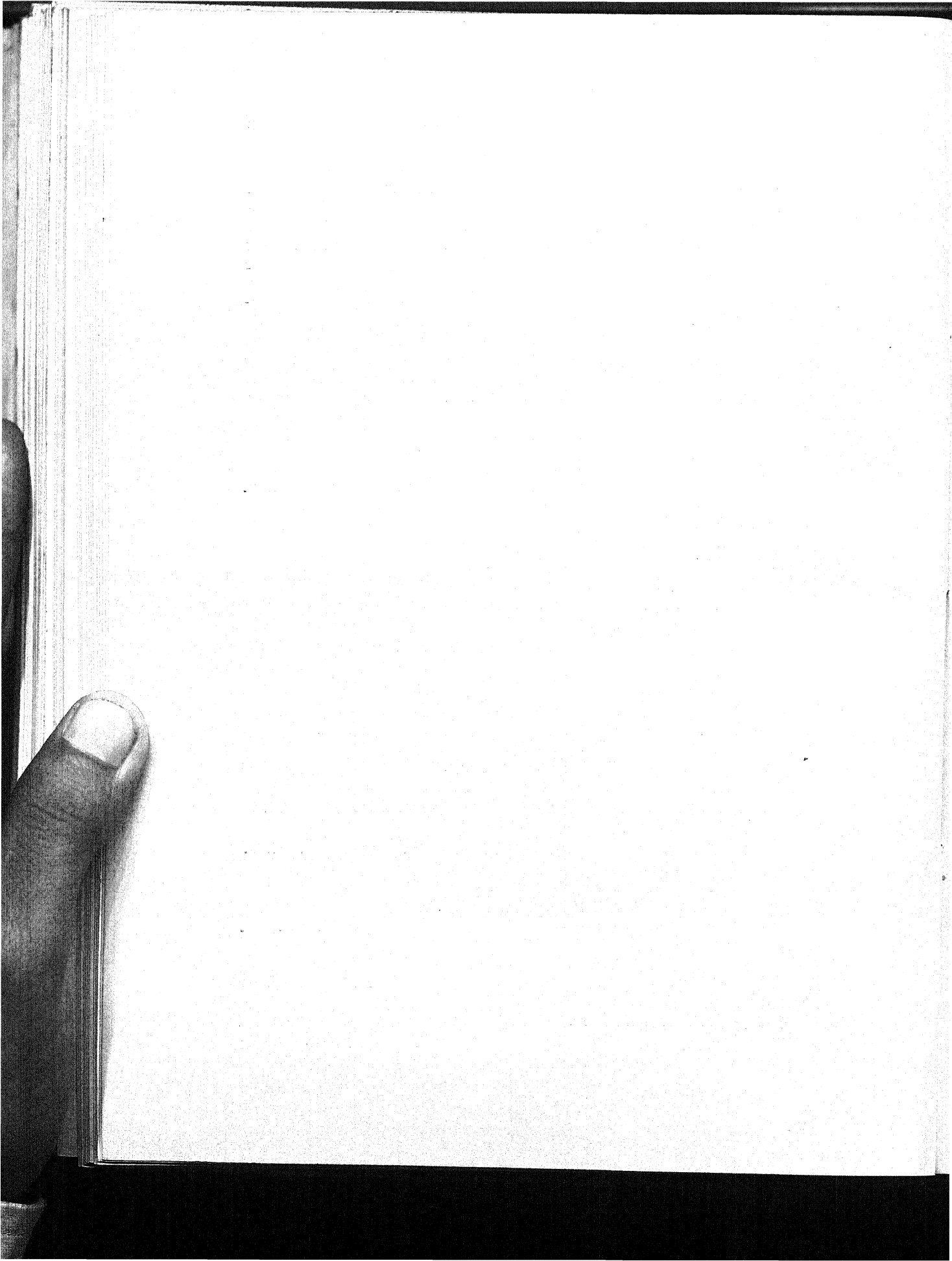

EXPERIMENTS ON THE EFFECT OF X-RAY ON *PENNISETUM TYPHOIDEUM* RICH., *NICOTIANA TABACUM* LINN. AND *BRASSICA JUNCEA* HK. f & T

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I. Introduction

Since Muller's [1927] important work, several investigators have studied the effect of X-rays on plants and animals. Species of *Nicotiana* and *Drosophila* are among the materials frequently used for irradiation experiments. Treating five species of *Nicotiana* with X-rays and radium, Goodspeed and Olson [1928] observed that the effect produced in all the species was that the progenies showed variation, some of the variations resulted from disturbances during mitotic and meiotic divisions and many of the variant characters in the progeny of X-rayed plants were inherited. Later Goodspeed [1929] had found that the effect of X-rays on *Nicotiana sylvestris* was similar to that on *N. tabacum* in producing variable quantitative chromosomal alterations which gave rise to series of by-products in later generations. Stadtler [1930] working on barley and maize found that the rate of induced mutation in germinating seeds was four times as much more as those in dormant seeds. When the dose applied to the dormant seeds was doubled the rate of mutation approximately doubled. The double dose applied to germinating seeds was almost completely lethal in effect. Increase in moisture

content and variations in temperature had no effect on rate of induced mutation. The gene mutations obtained by Stadtler were all of recessive type. Chromosomal reorganization observed by Goodspeed in tobacco was also observed by Stadtler in the case of barley and maize. Goodspeed and Avery [1934] obtained from the progeny of a single X-rayed female gamete of *N. tabacum* fourteen types. Seven of these were pure breeding types and the remaining seven not established as pure. These types differed from one another and the control in habit, form of leaf, flower and capsule, and in colour of leaf and flower. Chimeral effects as a result of exposure to high frequency waves were observed in barley and maize by Stadtler and in tobacco by Goodspeed. By subjecting the seeds of broad bean, barley and mustard to same condition and dosage which resulted in different effects, Patten and Wigoder [1929] concluded that a specific dose is required for each. Stunting followed in all cases where plants growing for twenty-four hours were rayed. Difference in sensitiveness between shoot and root was noted in barley. With the dosage used the only detrimental effect observable in mustard was the failure of side roots to develop. Shull and Mitchell [1933] working on wheat corn, oats, etc., found that brief exposure to X-rays produced from high voltage and low ampereage had a stimulative effect on plant growth. Using stronger dose of 300 r-units Moore and Haskins [1934] observed physiological variation and abnormalities including dwarfing, delayed growth and fasciation in leaves of cotton, tung-oil and other plants. Ramiah and Parthasarathy [1936] obtained an ageotropic mutant in rice by exposure to weaker dose. Singh and Choudhri [1935] noted that exposure of dry seeds of *N. tabacum* to X-rays for one minute had the effect of inducing physiological, morphological and chemical variations.

The authors have made no attempts to review here all the previous work done on X-raying plants but have referred to literature that was accessible to them.

II. Material and method

The first attempt to study the effect of irradiating crop plants with X-rays in Bombay Province was made by Dr. W. Burns in August 1931 and the work was later continued by the present authors. Through the courtesy of Major Chambers, Specialist in Radiology, the plants were rayed at the Connaught Military Hospital, Poona, for the first two years and later with the permission of the authorities the raying experiments were continued at the David Sassoon Hospital, Poona, for the remaining period. Because of the limited facilities available and because of the clinical purposes for which the X-ray apparatuses were used in the hospitals the irradiation experiments had to be restricted in scope and they could not be carried out to their conclusion.

Bajri (*Pennisetum typhoideum*), the bulrush-millet, mustard and tobacco were the materials used for the experiments. The result of each of these plants is discussed separately.

(*a*) *Bajri* (*Pennisetum typhoideum*)

The difference in time of maturity of the male and female flowers of *bajri* inflorescence was found suitable for irradiating the male and female gametes separately. The receptive stigmas are first extruded, followed a couple of days later by the first wave of mature anthers. The second wave of anthers ripens and becomes extruded from the glumes still a few days later. A few plants of a pure strain 54 of *bajri* were grown in earthen pots and when the earheads were ready for treatment, were removed to the hospital for raying. The male and female gametes were subjected to X-rays by exposing the ovaries and anthers respectively. The ovaries and anthers were separately rayed and pollination effected after the plants were brought back to the garden. The plant materials were rayed with a metalax water-cooled tungsten target X-ray tube. The dose and the period of exposure to which the earheads were subjected is given in Table I.

TABLE I

Dosage used for raying the ovaries and anthers of P. typhoideum

Experiment No. 1, 1931

——	K. V.	M. a.	Time of exposure in minutes	Target distance in inches
Dose 1	60	5	5	14
Dose 2	50	5	5	30 c. m. s.
Dose 3	62	5	5	14
Dose 4	45	5	5	14

The above doses were all applied without the use of a screen. The only two variables were distance and K. V., the other conditions were common to all. The pollination effected between rayed and unrayed material and the number of grains set in each earhead is given in Table II. The earheads were protected by paper bags and the grains produced were sown in the following season. For two generations selfed progenies of the irradiated plants were kept under careful observation.

TABLE II

Condition of the material used for pollination and the number of grains set

Earhead No.	Stage of development and treatment given		No. of seeds set	Remarks
	Ovary	Anther		
1 . .	mature unrayed .	mature rayed .	491	
2 . .	Do. . .	Do. . .	0	
3 . .	Do. . .	Do. . .	159	The average number of grain produced in a single earhead of *P. typhoideum* naturally grown is 1204.
4 . .	Do. . .	Do. . .	150	
5 . .	Do. . .	Do. . .	698	
6 . .	Do. . .	Do. . .	16	
7 . .	Do . .	immature rayed .	44	
8 . .	Do. . .	Do. . .	1099	
9 . .	mature rayed .	mature unrayed .	95	The average for rayed earheads is 381 grains.
10 . .	immature rayed .	Do. . .	1202	
11 . .	mature rayed .	Do. . .	248	
		Total .	4202	

From Table II it is observed that in one instance no seeds were set at all and in many setting is very poor. Only in two instances out of eleven the setting is normal. The seeds from the eleven earheads were sown in ear to row cultures in the following season.

The result of sowing showed that only in one instance (earhead No. 6 of Table II)—grains produced by pollination of unrayed earhead with rayed pollen—there was total failure of germination. In all other cases there was no indication of any adverse effect produced as a result of raying. The germination percentage of grains in all cases including the only two controls provided was exceedingly low. Provision of insufficient controls had made it difficult to draw definite conclusions. Except for the poor setting of grains in the majority of plants either of whose gametes were rayed (Table II) and the low percentage of germination observed while trying to raise the F_1 generation, no other abnormal or off-types could be noticed in any of the cultures of plants raised from treated materials.

Table II shows that in the majority of instances of earheads with either the male or female part mature and subjected to X-rays, the grain setting has been deficient as compared with the average number of grains produced in earheads grown under natural conditions.

In 1932 in addition to the exposure of ovaries and anthers to X-rays, germinating seeds of *P. typhoideum* were also rayed. The seeds treated were previously soaked in water for twenty-four, forty-eight and seventy-two hours. The following doses were applied to seeds of each of the three different periods of soaking as well as to ovaries and anthers.

TABLE III

Frequency of X-rays to which soaked seeds and earheads of P. typhoideum *were exposed*

Treatment No.	Distance in inches	Filter	K. V.	M. a.	Exposure in minutes
1 . . .	12	*Nil.*	50	5	10
2 . . .	12	*Nil.*	50	5	15

Since the X-ray apparatus at the hospital was small and not powerful enough, only few grains could be rayed at a time. Therefore, the data presented is that based on a small population and is not capable of being subjected to too critical statistical interpretation. The result of plants raised from rayed germinating grain is given in Table IV.

TABLE IV

Difference in growth effect between the progeny raised from soaked X-rayed and control grains of P. typhoideum.

Character studied	Rayed	Control	Difference
Yield of grain in grams	7·7	10·3	=2·6
Height of plant in feet	4·7	4·9	=0·2
Number of tillers per plant . . .	3·6	4·5	=0·9
Length of earheads in inches . . .	5·5	4·8	0·7

From Table IV it will be observed that plants raised from rayed grains show slight weakening as compared with the control except for the character of length of the earheads. No difference in result was observable between

grain soaked for different number of hours and later subjected to X-rays for varying periods. Since application of stronger doses was impracticable, further work on raying *P. typhoideum* had to be discontinued.

As in the case of earheads of *P. typhoideum* treated in 1931 so also in those rayed in 1932 the setting of grain was poor. The untreated controls produced normal well-set earheads. The grains that were set in rayed earheads when sown gave rise to normal progeny which resembled the controls for all characters. The result, therefore, of X-raying earheads of *P. typhoideum* to variations in unfiltered rays produced from 45 to 62 K. V., 5 M. a., at twelve to fourteen inches target distance from five to fifteen minutes had neither deleterious nor stimulative effect.

(*b*) *Mustard* (Brassica juncea *Hk.f. & T.*)

The apparatus used for X-raying mustard seeds was Victor Wanz Junior fitted with a coolidge air-cooled tungsten target X-ray tube. The dosage applied was 80 K. V., 5 M. a., target distance three inches, without filter. Dry seeds of mustard were exposed for nine minutes by three intermittent application of three minutes each since it was not possible to give continuous application.

The rayed seeds were sown in plots with suitable controls. The rayed and control materials were replicated seven times by A. B. B. A. method. The result of observations of the plants from rayed seeds is given in Table V.

TABLE V

Difference between rayed and unrayed dry seeds of mustard

Observation	Rayed material	Control	Difference	Significance of difference
Average No. of fruiting branches	7·67	8·40	=0·73	Not significant
Height in feet . . .	5·23	5·29	=0·06	Do.
Seed yield in grams. . .	4·13	5·73	=1·60	Do.

Although the above figures show that difference between the rayed and unrayed is not significant for any of the character studied yet a slight weakening of the plants raised from dry seeds exposed to X-rays for nine minutes is clearly noticeable.

(*c*) *Tobacco* (Nicotiana tabacum *Linn.*)

Both dry and soaked seeds of tobacco were exposed to same frequency of rays as that for mustard and the duration of exposure was limited to three minutes. The rayed seeds were sown in plots along with controls replicated

five times by A. B. B. A. method. The results of observations made on certain characters are given in Tables VI and VII for soaked and dry seeds respectively.

TABLE VI

Difference between rayed and unrayed soaked seeds of tobacco

Characters studied	Rayed material	Control	Difference	Significance of difference
Average No. of leaves per plant .	10·12	11·14	=1·02	Significant
Height of plant in inches . .	2·63	3·06	=0·43	Do.
Average leaf factor of biggest leaf	76·71	96·28	=19·57	Do.

The above comparison between the plants from soaked seeds exposed to X-rays and controls shows that the difference in respect of all the three characters studied is significant. Soaked seeds have, therefore, showed a general but significant weakening.

TABLE VII

Difference between rayed and unrayed dry seeds of tobacco

Characters observed	Rayed material	Control	Difference	Significance of difference
Average No. of leaves per plant .	9·91	10·51	=0·60	Not significant
Average height of plant in feet .	2·74	2·86	=0·12	Do.
Average leaf factor of biggest leaf	82·17	92·66	=10·49	Do.

From the figures given in Table VII it is observed that in the case of dry seed of tobacco the same treatment as that given to soaked seeds had slight but not significantly weakening effect. A comparison of the results of soaked and dry seeds of tobacco exposed to the same duration and dosage of X-rays shows that the deleterious effect observable in the weakening of plants is more pronounced in the case of soaked than dry seeds.

In 1934 the previous years' experiments were repeated but owing to a severe attack of damping-off disease both the control and rayed material were totally lost. These experiments could not be continued any further since the only apparatuses that could be employed were those in hospitals used for clinical work and were not powerful enough to induce changes abnormal in character.

A comparison of the effect of X-raying the aforementioned three crops seems to indicate that the dosages used have not produced any deleterious effect in any of them except one. Even the stronger dose used in the case of dry seed of mustard (80 K. V., 5 M. a., 3 in. distance and 9 minutes exposure) as compared with others has not produced any weakening while the same dosage used for only three minutes produced significant weakening effect on soaked seeds of tobacco. It was observed that the power of germination of rayed seeds, whether dry or soaked at the time of treatment, was generally affected. Treated seeds showed poorer germination as compared with the control. In Table VIII is given in a summarised form the results of raying the three crops used in these experiments.

TABLE VIII

Summary of results of raying given in tabular form

Crop	Condition of part rayed	K. V.	M. a.	T. distance in inches	Exposure in minutes	Result
Mustard .	Dry seed .	80	5	3	9	No appreciable weakening
Tobacco .	Dry seed .	80	5	3	3	Weakening not significant
Tobacco .	Soaked seed	80	5	3	3	Significant weakening
Bajri .	Soaked seed	50	5	12—14	5—15	No appreciable weakening
Bajri .	Earhead .	50	5	12—14	5—15	No appreciable weakening

III. DISCUSSION

Stadtler [1930] observed in the case of barley and maize and Goodspeed [1929] in the case of tobacco that soaked or germinating seeds were more adversely affected by X-rays than dry seeds. This is confirmed by the results obtained on raying mustard and tobacco. Nine minutes exposure had no deleterious effect on dry seeds of mustard while the same dose applied for three minutes in the case of tobacco had no effect on dry seeds but significantly weakening effect on soaked ones. Patten and Wigoder [1929] found that specific doses were necessary for different crops to induce changes. The result of the three crops used in these experiments lends support to this conclusion.

Singh and Choudhri [1935], exposing *Nicotiana tabacum* seeds to minute dose of X-rays for one minute, were able to induce physiological, morphological and chemical variations conducive to accelerated metabolic activity of the

plant, while the authors using the same material (*N. tabacum*) but slightly stronger dose obtained a very different result, *viz.*, a general weakening of the plant. Shull and Mitchell [1933], in whose experiments stimulative effect resulted in seedlings treated with mild dose of X-rays, state that deleterious effects are consistently obtained when unfiltered radiations are used. This explains the difference between the results of Singh and Choudhri [1935] and the present authors. In the latter's case no filter was used and consequently whatever beneficial or stimulating effect there might have been, was masked by the deleterious effect produced from unfiltered rays.

Moore and Haskins [1934], Ramiah and Parthasarathy [1936], and Singh and Choudhri [1935] irradiated dry seeds ; Shull and Mitchell [1933] Noguchi [1935] and Benedict and Kersten [1934] treated soaked seeds. These workers, however, have not compared the results of X-raying dry and soaked seeds of the same plant. The authors tried the effect of unfiltered X-rays on both dry as well as germinating seeds of *N. tabacum*. The difference in effect between these two treatments was that in the case of plants raised from irradiated dry seeds the general weakening was not significant as compared with the controls while for those plants raised from irradiated soaked seeds the difference due to weakening was significant. From the results it may be suggested that unfiltered rays produce more deleterious effect in the case of soaked than dry seeds. These results somewhat compare with the results of Goodspeed who observed that seeds of *Nicotiana* were resistant and seedlings susceptible to effect of high frequency radiation. Stadtler [1930] found that dry seed could stand ten times the dose to which germinating seeds were subjected to.

In order to induce mutations it appears that strong dosage is necessary. None of the doses applied proved lethal to the millet, mustard or tobacco used in the experiments.

IV. Summary

(1) The effect of X-raying ovaries and anthers of *P. typhoideum* with a weak dose of unfiltered X-rays produced neither deleterious nor stimulative effect.

(2) Germinating seeds of *P. typhoideum* exposed to the same dosage of X-rays as the ovaries and anthers resulted in the weakening of the progenies raised. But the weakening was not statistically significant.

(3) Mustard and tobacco seeds when rayed showed a general loss in vigour of growth. The weakening was significant in the case of plants raised from irradiated soaked seeds and not significant in the case of those raised from irradiated dry seeds.

Acknowledgeents

The authors wish to express their thanks to Dr. W. Burns for initiating the work and to Mr. D. B. Barve and Mr. S. R. Godbole in rendering assistance during the early stages of the work.

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REVIEWS

Statistical Technique in Agricultural Research. By D. D. PATERSON (First Edition—McGraw-Hill Publishing Company, Aldwych House, London, W. C. 2). Pp. 263. Price 18*s*.

THIS addition to the already considerable number of commentaries on statistical method is welcome, as the examples selected are simple and illustrative of statistical principles.

Chapter I deals with general principles, the need for the reduction of data, calculation of mean, sum of squares and standard deviation [why the sum of squares should be divided by (n-1)], the principle of normal curve of errors, the principle of statistical significance and Fisher's t test. Short methods of computation in each case have been illustrated in a popular way. The author might have mentioned at this stage the idea of " Fiducial limits " introduced by Fisher.

Chapter II deals with the analysis of variance, and explains in a routine way how to calculate the sum of squares and the variance due to each item. Fisher's z test has been well explained, but the author might have stressed that when some treatment differences are considerably larger than others, it is necessary to separate " the errors " into different sets, instead of using a combined analysis and have a single " error " as a basis for comparison.

Chapter III deals with ' Goodness of Fit ', though as a logical sequence, it should have preceded the analysis of variance. The fact that χ^2 and t distributions are special cases of that of z—a fundamental idea in dealing with these distributions, has not been stressed in this publication anywhere clearly. The examples given for illustrating the χ^2 test are well known, being commonly employed in preparing contingency tables connected with genetical problems ; but it would have been better to refer in detail to the additional aspect of χ^2 and to the correction necessarily made when frequencies are discontinuous or are very small which often happens at the tail end of the distribution.

Chapter IV dealing with "diagrams" is scanty as it has not touched upon any cases of asymmetrical curves.

Chapters V and VI deal with correlation and regression respectively, their separation is hardly necessary. The idea of regression in view of its increasing importance should have been given greater prominence.

Chapters VII to IX dealing with new designs of Field Experiments are of great interest to the agricultural experimenter. The author has rightly stressed that the first essential for good experimentation, which is often forgotten, is sound crop husbandry, *i.e.*, a detailed knowledge of the crop, soil, manurial requirements, cultural technique, etc. Then the site selected for the experiment should be as uniform as possible. The nature of experimental problems should be clearly defined, and the treatments should be so chosen as to have a common basis for comparison and as to give fairly wide differences in yields between them. Every agronomist now knows that for a valid interpretation of his experimental results, the size of plots, their arrangement and shape, number of replications, all contribute their share and that even in adopting the usual randomised-block method or Latin square, the greatest care in design is necessary to obtain a valid estimate for error.

Chapter VIII deals with serial and perennial crop experiments. The author has treated the subject in a popular way. It should, however, have been emphasised that for such a combined analysis of variance it is necessary, that error variances in the several seasons should not differ significantly if there should be any such difference the precision in the several seasons should be equalised by suitable weights. With regard to designs for perennial crops (such as tea or fruits), the author has rightly stressed that each plot should contain at least ten trees and that suitable guard row should be provided for (double row or single row depending upon the field available).

Chapter IX deals with some of the latest designs recently issued from Rothamsted and on which F. Yates has already published his bulletin (Imperial Bureau of Soil Science—Technical Communication No. 35—The design and analysis of Factorial Experiments). The analysis of a complex experiment, and that of the split plot design and the analysis of covariance have been explained in detail, but only a brief reference has been made to the "confounded layouts" possible in 2^3 and 3^3 designs.

This book will help towards a clear understanding of modern statistical ideas and will be found useful by the agricultural experimenter for whom it is meant.

Introduction to the Botany of Field Crops. 2 Volumes. (South African Agricultural Series, Vol. XVI). By J. M. Hector, Professor of Agricultural Botany, University of Pretoria. Pp. 1127, with 448 Text-figures and 88 Tables. Published by the Central News Agency Limited, Johannesburg, South Africa. Price £3 10*s.* 0*d.* net per set of 2 Volumes (postage 2*s.* extra).

The author in these two volumes has presented in a co-ordinated form the fundamental facts and principles relating to the structure, functions and life-relations of plants. A considerable advance has been made in the knowledge of botany of cultivated plants during the last two decades and investigations on a number of new lines have been undertaken. The literature during this period has also become extensive and widely scattered. The necessity for collecting and co-ordinating this information is manifest and the author has attempted to meet that demand. The study of structure being fundamental to a proper understanding of all other aspects of plant study, morphology is emphasised throughout. But since structure has significance mainly in terms of function, the principal physiological processes are also given a considerable attention. To obtain a fuller understanding of the field crops, approach has also been made from other angles, *i.e.*, cytogenetics, ecology and systematics. It is only by so doing that it is possible to understand a living creative organism. It not only gives a more thorough insight into the behaviour and reactions of plants in the field but leads to a better understanding of the potentialities of crop production.

The book is divided into two volumes —Volume I dealing with cereals and Volume II with other crops. The division, however, is not quite so absolute since Volume I also deals with non-cereal crops such as sugarcane. The examples of plants selected by the author for demonstrating various phenomena are taken from field and garden crops. In the description of plants generally the structure and functions of the vegetative organs of plants precede that of the organs concerned with reproduction. The seed development in most cases is well explained and fully illustrated but the conditions favourable to germination of seed are not stated. A description of the conditions required for successful germination of seeds of crops like sugarcane, tobacco, lucerne, berseem, etc. would have been useful. In cereals a statement of comparative nutritional value of millets and other grains would have greatly enhanced the value of the description of these crops. The chapter on maize, however, is comparatively more complete giving the economic uses of maize and food constituents of the grain.

One would normally not expect results of agronomic and fertiliser trials on the growth of plants in a botanical treatise of this nature but, since the author has given such results in the case of cotton, it would have been just

as well to give similar results for other crops to make the book more complete. In relation to Indian work, references to recent investigations on crop husbandry in different schemes of the Imperial Council of Agricultural Research could have been usefully added to bring the knowledge up-to-date.

The author has rightly paid greater attention to those Natural Orders which include important agricultural crops, for instance, the Leguminosae and the Malvaceae, and has scantily dealt with those such as the Compositae which, though large, are not so important agriculturally. The Leguminosae occupies the largest space covering 167 pages with the Malvaceae coming next with 114 pages. The description of these two Orders is of the nature of a running commentary of botanical investigations on the different families. The chemistry of nitrogen fixation by the different plants of the Leguminosae and the differentiation on morphological and physiological grounds of organisms causing nitrogen fixation are useful contributions to knowledge. The chapters on the Solanace, the Cucurbitaceae and Liliaceae give a description of plants, the structural knowledge of which will be found useful by those interested in vegetable gardening. In dealing with potato, the author has described the quality in various types. Useful findings in regard to the effect of light on germination of potato are recorded but perhaps an account of relative usefulness of practical methods of preservation of seed potatoes would have been a more useful contribution. In taxonomic studies the author has rightly laid stress on the necessity of a change in the outlook of methods of classification and advocates the principles of adoption of new units of differentiation based on genetic variability. This principle has already been expounded by the Imperial Council of Agricultural Research in the paper entitled " The Description of Crop-plant Characters and their Ranges of Variability—(*i*) in Cotton and (*ii*) in Rice " [*Ind. J. Agric. Sci.* Vol. VIII, Part V, p. 567.]

The book is comprehensive in scope including material from all the divisions of botany. It is written neither merely as a text book in the accepted meaning of the term nor is its subject matter intended for any particular course. It forms a useful compendium of botanical investigations on cultivated crops and will thus be found useful by botanists and agronomists undergoing post-graduate training. The author has given references to relevant literature containing detailed accounts of various researches mentioned in the text. At the end of each chapter there is a comprehensive and useful bibliography. The author's index, plant index and general index at the end of the book enhance its utility. The book is profusely illustrated and most of the illustrations have been selected from the original publications. Mention may also be made of a few slips and printing mistakes which require to be rectified. By way of example, on page 287, the figure is intended to show lemna colouration but there are no colours in it ; on page 623 " Sabnis and Phatak " are wrongly spelt as " Sabris and Phatax " On page 899, " Lyallpur " is mis-spelt as " Syallpur " and " Afzal and Iyer " are noted as " Afzal and Tyer ".

The book is a useful contribution to the knowledge of science of applied botany and deserves to be read by every student interested in that science and should be on the shelf of every library as a reference book. (R.L.S.)

ORIGINAL ARTICLES

NITROGEN FIXATION IN RICE SOILS

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(With one text-figure)

INTRODUCTION

IN ordinary farming practice rice is grown in the Konkan without the addition of manure or fertiliser to the soil. Sahasrabuddhe and Abhyankar [1936] have shown that cultivated rice soils at Karjat contain more nitrogen than the uncropped soils during the growing period of rice crop. Sahasrabuddhe [1936] later confirmed these findings by laboratory experiments and was further able to show that rice soils carry an inoculum which has the power of fixing nitrogen and that this fixation of nitrogen is helped by the presence of growing roots of rice plants. He did not, however, attempt an elucidation of the nature of this inoculum, which, as has been shown in the following pages, consists of free-living nitrogen-fixers.

Starkey [1929] cites several workers who have shown that tobacco, corn, mustard and some crucifers are benefited by the presence of *Azotobacter* in the soil. He [1931] has also reviewed the evidence indicating that the bacterial life is greatly enhanced in cultivated soils and is virtually at a standstill in fallow. Lipman and Brown [1907], however, failed to show any definite increase in the nitrogen-content of the soil which had been inoculated with *Azotobacter vinelandii* and *A. beijerincki* and cropped with oats, corn and rye in succession. On the other hand, Stranak [1910] showed that potatoes, grain and beets grew excellently in soil inoculated with *Azotobacter*. Although these results are conflicting, yet it seems possible that under suitable conditions of growth *Azotobacter* can fix appreciable amounts of nitrogen.

Sen [1929] suggested the possibility that nitrogen-fixing organisms may live symbiotically in the roots of rice plants, whilst Viswa Nath [1932] is of the opinion that the rice plant itself has the power of fixing atmospheric nitrogen. Recently De [1936], working with a mixed culture of algae and bacteria, came to the conclusion that the fixation of nitrogen in rice soils under waterlogged conditions is an algal process, although he was unable to determine whether the fixation was brought about by the algae themselves or by algae working in symbiosis with bacteria. On the other hand, Bristol and Page [1923] have shown that pure cultures of algae do not possess the power of fixing elementary nitrogen, though Allison and Hoover [1935] have recently claimed this ability for certain *Nostoc*.

The conflicting evidence on the nitrogen recuperation of cultivated soils led us to the re-examination of the problem by direct experiments with pure cultures of the organisms isolated from rice soils at Karjat.

Experimental methods and results

Rice plants with soil about their roots were collected during the rainy season in July 1936, from different plots on the Rice Breeding Station at Karjat ; the soil was submerged at the time when the plants were removed. Isolations from soil samples were made on Ashby's agar using dilution method. The plates were incubated at room temperature, varying from 25° to 27°C. Colonies of bacteria began to appear in the plates after 48 hours, and a majority of these colonies turned dark with age. These dark colonies were further purified and the organism was then grown on slants of Ashby's agar.

For the purpose of inoculation a two days' growth of the organism on Ashby's agar was invariably used. Inoculation was done by scraping growth from agar slants with a sterile needle and transferring it to flasks containing 50 c.c. of Ashby's solution.

Experiment I.—The organism was isolated from samples of submerged soil collected in the immediate proximity of the roots of growing rice plants at Karjat. The transfer of the organism was made into culture flasks each containing 50 c.c. of Ashby's solution, in some of which rice plants were also grown. The experiment lasted for 30 days after which total nitrogen was determined by Ranker's [1927] modification of the salicylic-thiosulphate method. Two series of tests were made in duplicate at different times, and the results are summarised in Table I.

The results show that all the four cultures of the organism isolated from soil samples from Karjat can fix appreciable amounts of nitrogen and that isolate III (Is. III) is an efficient nitrogen-fixer. These results further bring out the interesting fact already reported by Sahasrabuddhe [1936] that growing roots of rice plants markedly enhance the nitrogen-fixing capacity of rice soils.

TABLE I

Amounts of nitrogen fixed by organism isolated from rice soil

Ashby's solution containing	Total nitrogen in mg.			
	Series A		Series B	
	Individual flask	Average	Individual flask	Average
Isolate 0	1·24 1·22	1·23	.. ..	..
Isolate I	1·37 1·41	1·39	0·9 0·9	0·9
Isolate II	1·42 1·48	1·45	0·965 0·965	0·965
Isolate III	1·64 1·62	1·63	0·965 0·965	0·965
Isolate 0+9 rice plants	2·982 3·038	3·01	.. ..	..
Isolate I+9 rice plants	3·097 3·183	3·14	2·576 2·700	2·638
Isolate II+9 rice plants	3·09 3·07	3·08	2·856 2·752	2·804
Isolate III+9 rice plants	3·712 3·648	3·68	3·221 3·285	3·253
9 rice plants alone	1·432 1·408	1·42	1·538 1·522	1·53

In view of the fact that the nitrogen-fixing power of isolate III is quite high, it has been used as inoculum in the experiments that follow.

Experiment II.—There is a definite indication from Experiment I that the association of rice plants with the organism enhances its normal activity to fix nitrogen. It is not known, however, whether the introduction of sterilised soil into the culture flasks exerts a similar beneficial effect on the organism. An experiment was therefore made to study the effect of the association of rice plants and soil on the activity of the organism to fix nitrogen.

Seeds of Kolamba variety of rice were obtained from Karjat and disinfected in 1 in 500 solution of mercuric bichloride for 15 minutes. The seeds were then washed in sterile water and transferred aseptically to sterilised glass chambers lined inside with sterile, damp filter paper. After germination of seed the seedlings were allowed to grow in the glass chambers kept at 32°C. for eight days before removing them to culture flasks.

Five sets of culture flasks each containing 50 c.c. of Ashby's solution were prepared and treated in the following manner :—

(1) Inoculated with Is. III

(2) One gram of sterilised rice soil was added to each of the culture flasks in this set, of which half the number was inoculated with Is. III

(3) Nine rice plants were grown in each of the culture flasks in this set, of which half the number was inoculated with Is. III

(4) In one group containing half the number of culture flasks included in this set, nine rice plants were grown in each culture flask to which one gram of sterilised rice soil was added ; the second group was similarly treated but was inoculated with Is. III and

(5) Same as in the second group of (4) above except that in half the number of culture flasks in this set roots of rice plants were introduced into Ashby's solution for ten minutes and in the other half rice plants were grown for ten days, before inoculating the culture flasks with Is. III.

At the conclusion of the experiment after 30 days the total nitrogen was determined from duplicate samples of each culture flask including the rice plants. The results given in Table II are of two series done at different times.

The results presented clearly show that the organism, in association with rice plants or rice soil or both, is able to fix larger amounts of nitrogen than it does in the absence of such association. Rice soil which has been sterilised does not possess the power of nitrogen fixation since the small difference in its nitrogen content (0·863—0·320 mg.) and that of Ashby's solution (0·320 mg.) may be due to the amount of nitrogen already present in the soil. However, when the culture flask containing sterilised soil is inoculated with the organism, 2·838 mg. (3·733—0·895) nitrogen is fixed. It is not clear how soil can stimulate the organism to greater activity unless it is assumed that this beneficial effect is due to certain minerals and salts such as phosphates supplied by the soil [Waksman, 1931]. Likewise, rice plants themselves are incapable of fixing atmospheric nitrogen, but in their presence the ability of the organism to fix nitrogen is greatly enhanced due probably to the excretion of growth-promoting substances from the roots. Further, in the presence of both soil and roots of rice plants as simulating conditions met with in the field, the organism is aroused to still greater activity as it can fix 5·611 mg. (8·200—2·589) or 6·610 mg. (8·827—2·217) nitrogen per 0·5 gram of mannite utilised in 50 c.c. of Ashby's solution. Another interesting fact

emerging from these experiments is that, when rice plants are allowed to grow in Ashby's solution before inoculation for a period longer than ten minutes, the growth of the organism is adversely affected with the result that the efficiency of nitrogen fixation decreases, *i.e.*, 2·776 mg. (5·365—2·589) nitrogen is fixed when roots of rice plants are introduced for ten minutes in Ashby's solution as against 1·087 mg. (3·676—2·589) nitrogen fixed when plants are grown for ten days.

TABLE II

Effect of association of rice plants and soil on the nitrogen-fixing power of the organism isolated from rice soil

No.	Ashby's solution containing	Total nitrogen in mg.			
		Series A		Series B	
		Individual flask	Average	Individual flask	Average
1	Is. III	1·688 1·710	1·699	1·416 1·404	1·410
2	1 grm. sterilised soil	0·863 0·863	0·863	0·895 0· 95	0·895
	1 grm. sterilised soil+Is. III	.. ..	..	3·712 3·754	3·733
3	9 rice plants	1·531 1·555	1·543	1·680 1·696	1·688
	9 rice plants+Is. III	3·321 3·357	3·339	3·415 3·349	3·382
4	1 grm. sterilised soil+9 rice plants.	2·602 2·576	2·589	2·230 2·204	2·217
	1 grm. sterilised soil+9 rice plants+Is. III.	8·245 8·155	8·200	8·800 8·854	8·827
5	1 grm. sterilised soil+9 rice plants (10 minutes)+Is. III.	5·255 5·475	5·365	.. ..	..
	1 grm. sterilised soil+9 rice plants (10 days)+Is. III.	3·662 3·690	3·676	.. ..	..

N.B.—Ashby's solution in these experiments contained 0·320 mg. nitrogen. Nine rice plants in these experiments contained about 1·2 mg. nitrogen.

Experiment III.—The previous experiment has shown that the organism in association with living rice roots is able to fix larger amounts of nitrogen. This experiment was therefore undertaken to ascertain whether any growth-promoting substance is contained in the living roots of rice plants or whether the stimulating effect is caused by increase in available energy due to the introduction of plant residues such as roots. For this purpose two groups of culture flasks, in duplicate, each containing 50 c.c. of Ashby's solution and

a gram of sterilised rice soil were set up; five c.c. of the extract prepared from the roots of nine of the rice seedlings grown under sterile conditions in Knop's solution was added to one group of flasks and choppings of roots of the same number of plants grown under identical conditions to another group of flasks. After twenty days the total nitrogen was determined from each culture flask, and the results are recorded in Table III.

TABLE III

Effect of rice root extract and choppings on the nitrogen fixing power of the organism

No.	* Ashby's solution+soil containing	Total nitrogen in mg.	
		Individual flask	Average
1	Root extract	1·910 1·709	1·809
	Root extract+Is. III	2·376 2·310	2·343
2	Root choppings	1·718 1·696	1·709
	Root choppings+Is. III	3·085 3·086	3·085
**3	9 rice plants growing	2·096 2·095	2·095
	9 rice plants growing+Is. III	6·987 6·004	6·495
**4	Is. III	2·549 2·439	2·499

* Ashby's solution+soil contained 0·874 mg. nitrogen.
** Controls.

In considering the results in the above table, the following points seem to be established :—

(1) The organism is stimulated to greater activity when grown in culture in association with living roots of rice plants as it can fix 3·996 mg. (6·495—2·499) nitrogen as against 1·625 mg. (2·499—0·874) nitrogen fixed by the organism without association. The amount of nitrogen thus fixed is more than when an extract of the roots or their choppings are added to the culture, thus indicating that the stimulation of the organism is brought about in some manner by the living roots and is not due to any substances present in the dead roots.

(2) The addition of the root extract to the culture flasks does not exert any stimulatory effect on the power of the organism to fix nitrogen since the amount of nitrogen thus fixed is almost the same as in the control; but some

slight beneficial effect is discernible when root choppings instead of the root extract are introduced into the culture flasks. This small increase in nitrogen fixation may be due to the organic matter serving as an additional source of energy.

Experiment IV.—In an earlier experiment indication was obtained that the time for which the living roots of rice plants are growing in Ashby's solution previous to inoculation is an important factor affecting the power of the organism to fix atmospheric nitrogen. To test this point further, a series of culture flasks each containing 50 c.c. of Ashby's solution and a gram of sterilised rice soil was prepared. Nine rice seedlings were grown in each of the culture flasks for periods varying from ten minutes to ten days and the flasks were subsequently inoculated with Is. III. The contents of the culture flasks were analysed for total nitrogen at the end of twenty days after the inoculation of each flask. The results are given in Table IV.

TABLE IV

Effect of growing rice seedlings in culture previous to inoculation on nitrogen-fixing power of the organism

Period for which rice seedlings grown previous to inoculation	*Total nitrogen in mg.	
	Individual flask	Average
10 min.	4·990 5·122	5·056
30 min.	4·294 4·442	4·368
3 hours	4·016 3·864	3·940
1 day	3·300 3·378	3·339
3 days	2·784 2·848	2·816
7 days	2·984 2·848	2·916
10 days	2·388 2·432	2·410
Control (with Is. III)	2·499 2·499	2·499

* Exclusive of nitrogen in nine rice seedlings removed previous to inoculation.

It is clear from the table that progressively less amounts of nitrogen are fixed by the organism in culture medium in which rice seedlings have previously been grown for ten minutes to ten days, although there is not much difference in the nitrogen contents of the culture flasks in which rice seedlings have been grown for three and seven days before inoculation. The growth of rice seedlings in culture flasks for ten days so changes the culture medium that it has a depressant effect on the organism which fixes less nitrogen than in the control. In other cases the association of the organism with the living roots stimulates its activity to a greater or less degree as seen from the amounts of nitrogen fixed.

A possible explanation of these results might be sought in a change in the reaction of the culture medium brought about by rice seedlings growing in it for varying lengths of time. The above experiment was therefore repeated with some modifications in the length of time for which the rice seedlings were grown in the culture medium. The reactions of the solution in each culture flask were determined electrometrically at the beginning and at the conclusion of the experiment. The results in Table V indicate that the solution becomes more alkaline as the period of contact of living rice roots with Ashby's solution increases.

TABLE V

Reaction of Ashby's solution in which rice seedlings were grown for varying lengths of time

Period for which rice seedlings grown previous to inoculation	*p*H	
	Initial	After removal of plants
10 min.	6·88	6·90
30 min.	6·88	7·16
3 hours	6·88	7·17
2 days	6·88	7·19
5 days	6·88	7·50
7 days	6·88	7·64
10 days	6·88	7·90

Experiment V.—This experiment was done to ascertain the *p*H of the culture medium favouring the most vigorous development of the organism. Ashby's solution (without calcium carbonate), to which one gram of sterilised rice soil was added, was adjusted to different *p*H by adding to it appropriate

quantities of $N/100$ hydrochloric acid and $N/100$ caustic soda to give a range of pH varying from 4·79 to 7·59 after the sterilisation of the medium. The culture flasks were subsequently inoculated with Is. III and incubated at room temperature for 20 days. The pH of the solution in each culture flask was determined at the conclusion of the experiment and also the amount of nitrogen fixed by the organism. The results are recorded in Table VI.

TABLE VI

Effect of different pH on the development of the organism

Initial pH	pH at conclusion of experiment	Total nitrogen in mg.
4·79	5·38	1·606
5·63	5·50	2·047
5·67	5·66	2·031
6·06	5·75	2·912
6·30	5·78	3·679
6·54	5·84	3·533
6·79	6·01	3·109
6·91	6·32	3·106
7·20	6·55	3·505
7·29	6·58	3·128
7·59	6·63	3·185
6·06 (Control)	5·84	0·975

The results show that the organism fixes the largest amounts of nitrogen at pH 6·3 to 6·54 ; on either side of this range the nitrogen-fixing power of the organism decreases although it is more adversely affected in the acidic than in the alkaline medium. The organism is able to produce acid by the utilisation of mannite in the medium as shown by the more acid reaction in almost every case at the conclusion of the experiment. This evidence, when considered with the data in Tables IV and V, clearly establishes the fact that the organism is most active in a slightly acid medium and, as the medium is rendered more alkaline by contact with growing rice seedlings, the activity of the organism to fix nitrogen suffers a decline.

Organism

The organism is a species of *Azotobacter*, and the following is a description of its morphology and cultural and physiological characteristics :—

Rods: 2·0 to 3·2 by 2·0 to 2·8 μ, occurring in pairs (diplococcus) and a few in packets (pediococcus). Motile. Encapsulated. Gram-negative. Brown to black pigment begins from the third day onwards. Pigment production begins earlier in thickly seeded plates, colonies buried in the medium not producing pigment.

Frazier's gelatin medium: No liquefaction.

Ashby's mannitol agar colonies: Glistening, opaque, circular, raised, smooth.

Neutral beef broth: No growth.

Litmus milk: Becomes clearer in seven to fourteen days.

Potato cylinders: Moderate, glistening, brown, slightly slimy growth.

The organism fixes atmospheric nitrogen and grows in a crude, black film on the surface of Ashby's mannitol solution; it produces slight acid but no gas when mannitol in Ashby's solution is replaced by saccharine, maltose, galactose, levulose, lactose, raffinose, glucose, salicin, dextrin, asparagin, amygdalin, glycerine and inulin.

Aerobic.

Optimum temperature about 26°C.

Habitat: Soil.

Periodicity

The main purpose of this study was to estimate the density of *Azotobacter*-like organisms in rice soils at Karjat in order to ascertain roughly the numbers occurring in any one year as part of a normal recurring cycle. The seasonal changes in numbers of the organism have an important bearing on factors affecting crop yield, and it was held to be probable that there existed a correlation between the numbers of non-symbiotic organisms of the type of *Azotobacter* in cultivated rice soils at Karjat and the nitrogen-content of the latter.

Beginning with August, composite samples were taken from the 2-4 in. layer of cultivated rice soil at Karjat and of fallow at intervals of about three weeks during 1936-37. Plate cultures of different dilutions were prepared from one-gram samples of soils, and a selective medium like Ashby's agar was used for culturing the organism. The relative incidence of different types of organisms was noted by an examination of the colonies, and the number of such bacterial colonies on a plate as resembled *Azotobacter* colonies was counted. In each culture flask the determination of total nitrogen including nitric nitrogen was done according to the method described in the "Official and Tentative Methods of Analysis" by the Association of Official Agricultural Chemists [1935]. For each determination five grams of dry

soil were taken and wetted with water previous to the addition of salicylic-sulphuric acid mixture. The results are recorded in Table VII and those for cultivated soil are also shown graphically in Fig. 1.

TABLE VII

Azotobacter-*like organisms in rice soil and fallow per gram*

Date of collection	Cultivated soil		Fallow	
	No. of colonies	Total nitrogen in mg.	No. of colonies	Total nitrogen in mg.
August 3, 1936 . . .	1,600,000	0·837	0	0·686
August 27, 1936 . . .	340,000	1·024	0	0·668
September 23, 1936 . .	56,000	1·170	0	0·674
October 10, 1936 . . .	41,000	0·930	0	0·718
October 27, 1936 . . .	2,000	1·009	0	0·780
November 21, 1936 . .	300	0·964	0	0·612
December 12, 1936 . .	1,000	0·989	0	0·674
January 2, 1937 . . .	0	0·710	0	0·892
January 30, 1937 . . .	0	0·776	0	0·729
February 21, 1937. . .	300	0·772	0	0·531
March 15, 1937	0	0·752	0	0·522
April 5, 1937 . . .	450	0·801	0	0·774
April 27, 1937 . . .	25	0·807	0	0·450
May 8, 1937 . . .	0	0·726	0	0·549
May 29, 1937 . . .	500	0·773	0	0·994
June 19, 1937 . . .	400	0·872	0	0·846
July 10, 1937 . . .	8,000	0·910	0	0·687
July 31, 1937 . . .	110,000	0·815	0	0·619
August 21, 1937 . . .	280,000	0·729	0	0·483

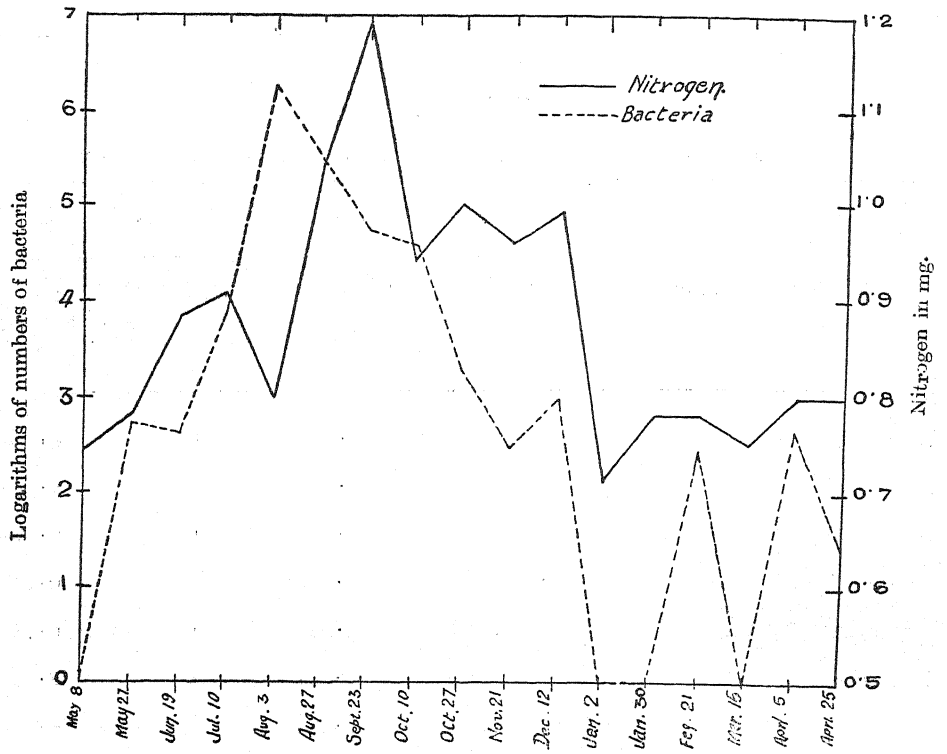

Fig. 1 Density of *Azotobacter* in and nitrogen content of rice soil in different seasons.

It will be seen from Table VII that fluctuations in the density of *Azotobacter*-like organisms in the rice soils are of such magnitude that numbers of these bacteria found in any one month cannot be accepted as representing the actual state of flora during other times of the year. These fluctuations are due to external climatic influences in the soil since the organism is very abundant during the rainy season from July to October when the rice crop is growing. After the monsoon the numbers of bacteria suffer a sharp decline until the organism becomes scarce and almost disappears from the soil in the cold season and during hot months. These seasonal fluctuations in microbial activity, though apparently due to climatic influences, may also have something to do with the growth of the crop since fallow does not support any population of *Azotobacter*-like organisms. The microbial activity in cropped soils is further reflected in an increase in the nitrogen-content of these soils as compared with fallow, more particularly in the rainy season. The presence of straw and its derivatives and large amounts of other organic matter as sources of energy for nitrogen-fixing bacteria will explain the great predominance of these organisms in cultivated rice soils and their complete absence from fallow lands.

SUMMARY

It has been shown that *Azotobacter* plays an important role in the nitrogen recuperation of rice soils at Karjat. The organism is stimulated to greater activity when associated with the growing roots of rice plants, which seem to cause an increase in its efficiency of nitrogen fixation by bringing about a change in the reaction of the medium in the vicinity of living roots.

The organism shows a marked periodicity due probably to external climatic influences in the soil as it is very abundant during the monsoon but suffers a sharp decline in numbers in the dry period following the rains until it becomes scarce and almost disappears from the soil in the cold season and during hot months. This microbial activity during the monsoon is reflected in an increase in the nitrogen-content of the rice soils. The seasonal fluctuations in microbial activity may also have something to do with the growth of the crop since fallow does not support any population of *Azotobacter*-like organisms.

ACKNOWLEDGMENT

In conclusion the writers wish to acknowledge the assistance rendered by Dr V. N. Gokhale and Mr. M. M. Kibe in analysing samples for their nitrogen-content.

Addendum

After this paper was submitted for publication, the present writers saw a note on "Nitrogen fixation by blue-green algae" by F. E. Fritsch and P. K. De [*Nature* **142**, 878. 1938] in which the latter state that they have isolated in pure cultures three species of *Anabaena* which have the property of fixing considerable quantities of nitrogen from the air. There was, however, no increase in the amount of nitrogen fixed when mixed cultures of *Anabaena* and *Azotobacter* were used, from which the authors conclude that *Azotobacter* plays "a relatively unimportant part in nitrogen fixation in the rice fields"—a fact which is contrary to the results reported in the present paper in so far as the soils of the rice fields at Karjat are concerned.

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THE STEM-BLACK DISEASE OF APPLE IN KUMAUN

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(With Plates XXXII and XXXIII)

Introduction

The stem-black disease of apple is caused by *Coniothecium chomatos porum* Corda. Strains of this fungus were found by Shaw [1918] and Butler [1919] to cause apple-cracking and branch blisters of apple trees in Kumaun. A similar stem-black disease of apple, though differing in symptoms from the one occurring in Kumaun, is described by G. H. Cunningham [1907] in his book ' Fungus Diseases of Fruit Trees in New Zealand '. It was first recorded there by Kirk [1907]. The disease is known in Australia, England and South Africa and in India it is known to occur in Baluchistan, Kurram Valley, Solan (Khattoo Fruit Orchards) and Kulu. It is now found in every orchard in Kumaun. The causal fungus usually attacks and kills the thick branches of apple trees. The death of a thick branch means considerably less apple crop and since the disease is present in all the orchards in Kumaun the economic loss due to it to the orchardists must be very considerable. A survey in the Government Orchards, Chaubattia in Kumaun, in 1936, showed that 60 per cent of the apple trees had stem-black. The death of the affected branches is hastened sometimes by the attack of *Botryosphaeria ribis*, the causal fungus of the stem-brown disease of apple. Other fruit trees are not known to be affected by the stem-black. The disease appears by the middle of July and is in its maximum virulence by the middle of August.

Symptoms

The disease starts at the pruned surfaces and proceeds downwards, forming a jet black streak, which slowly extends, surrounds the whole of the branch and ultimately kills it (Plate XXXII, figs. 1 and 2). In advanced stages cankers are formed (Plate XXXII, fig. 3). Thick branches are more susceptible than thin ones. The fungus was not observed to attack uninjured surface.

Morphology

An examination of a large number of transverse sections of the diseased portions showed that the fungus killed the tissue of the cortex down to the wood (Plate XXXIII, fig. 1). The hyphae were very short and were hardly different from the conidia. The conidia were not catenate ; they were irregular circiniform, muticate, often coalescent. The conidiophores and conidia were dark brown in colour (Plate XXXIII, figs. 2, 2*a* and 2*b*). The conidia measured $8 \cdot 4 - 36 \cdot 4\mu \times 5 \cdot 6 - 30 \cdot 8\mu$, the average being $16 \cdot 3\mu \times 11 \cdot 4\mu$. Their size was, thus, very variable. Chlamydospores were formed in nature (Plate XXXIII, fig. 4). Both conidia and chlamydospores germinated readily in tap water (Plate XXXIII, figs. 3 and 4*a*) within 24 hours. It was observed that about 5 per cent of them grew directly into germ tubes, while the rest burst and released a large number of minute, hyaline secondary spores. The latter, after a time, either produced buds or grew into germ tubes. The buds also germinated and produced germ tubes (Plate XXXIII, figs. 5 and 6). The secondary spores measured from $2 \cdot 8 - 7 \cdot 0\mu \times 2 \cdot 8 - 6 \cdot 3\mu$, the average being $4 \cdot 79\mu \times 4 \cdot 08\mu$ (Plate XXXIII, fig. 5). Rupturing of the conidia into secondary spores and their budding were also observed by Massee [1915] and he considered the secondary spores to be the potent source of fresh infection.

Isolation

The isolation of the causal fungus was made with single spores from diseased stems. The fungus was black in colour and very slow growing.

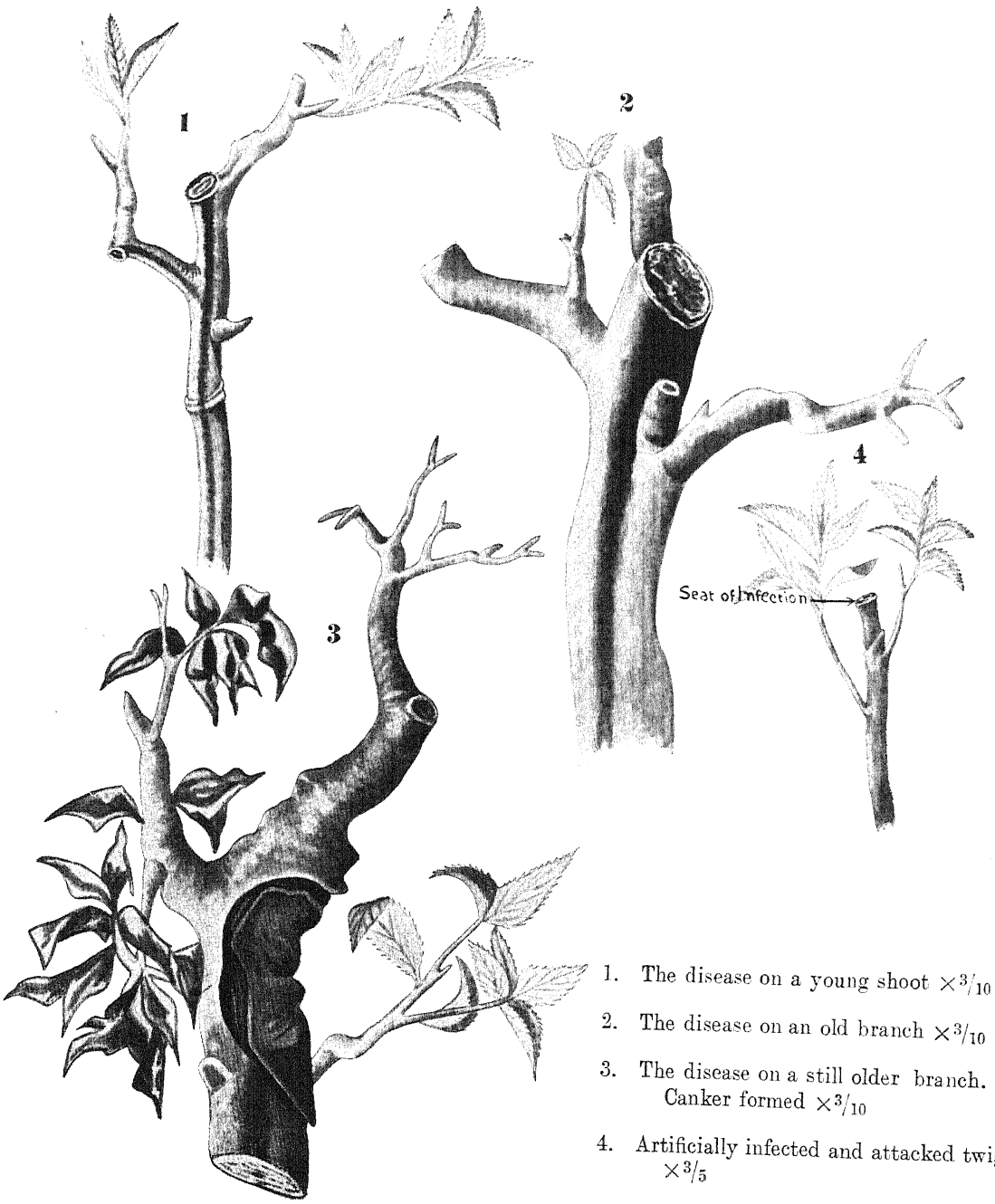
Pathogenicity studies

(*a*) A number of infection experiments were carried out by placing drops of spore suspension on uninjured surfaces of stem ; but in no case infection took place. When the surface was injured, however, the fungus readily penetrated and ramified in the tissues.

(*b*) On the 5th July 1935, three one-year old plants of apple, variety Delicious, were pruned and the cut surfaces were inoculated with the pure culture of the fungus. They were then covered with sterilised wet absorbent cotton and enclosed in cellophane bags. Two other one-year old plants of the same variety were similarly treated but were not inoculated. They served as controls. All the plants were kept inside a glass cage in which water was sprayed from time to time to maintain a saturated atmosphere. After seven days the cellophane bags were removed. Infection was noticed to have taken place in all the inoculated surfaces ; the controls remained unchanged.

Another experiment was similarly started on the 13th July with three plants of the variety Esopus Spitzenberg. The infection took place in all of them within seven days, except in the controls.

STEM-BLACK DISEASE OF APPLE CAUSED BY *CONIOTHECIUM CHOMATOSPORUM* CORDA



1. The disease on a young shoot $\times 3/10$
2. The disease on an old branch $\times 3/10$
3. The disease on a still older branch. Canker formed $\times 3/10$
4. Artificially infected and attacked twig. $\times 3/5$

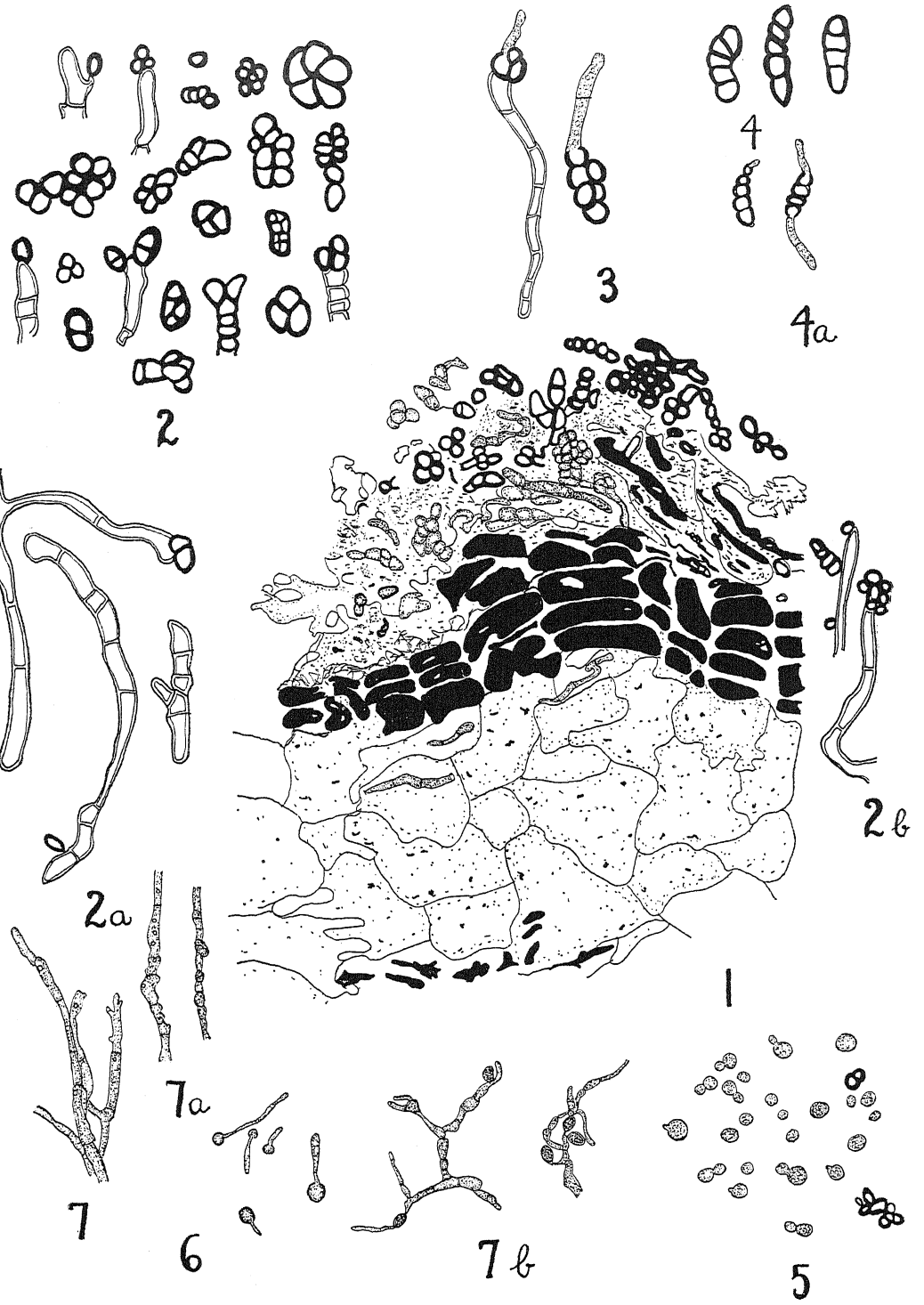

2
3
4
4a
1
2a
2b
7
7a
6
7b
5

PLATE XXXIII

FIG. 1. The transverse section, 6 μ thick of the bark of the apple stem showing the affected portion (×480)
FIG. 2. Conidia of the causal fungus *Coniothecium chomatosporum* (×480)
FIG. 2*a* and 2*b*. Conidiophores with conidia (×480)
FIG. 3. Germinating conidia (×480)
FIG. 4. Chlamydospores (×480)
FIG. 4*a*. Germinating chlamydospores (×480)
FIG. 5. A ruptured conidium with the released secondary spores. Some of the latter are budding (×480)
FIG. 6. Germinating secondary conidia (×480)
FIG. 7, 7*a* and 7*b*. Mycelium and chlamydospores in culture (×480)

PLATE XXXIII

Fig. 1. The transverse section, 6 μ. thick, of the bark of the apple stem showing the affected portion (×180)
Fig. 2. Conidia of the causal fungus *Coniothyrium clonuloporum* (×180)
Fig. 2a and 2b. Conidiophores with conidia (×180)
Fig. 3. Germinating conidia (×180)
Fig. 4. Chlamydospores (×180)
Fig. 4a. Germinating chlamydospores (×180)
Fig. 5. A ruptured conidium with the released secondary spores. Some of the latter are budding (×180)
Fig. 6. Germinating secondary conidia (×180)
Fig. 7, 7a and 7b. Mycelium and chlamydospores in culture (×180)

A third experiment was carried out with six seedling apple plants on the 5th of May of the following year. Two of them served as controls. Infection took place in ten days in all the inoculated plants. The first symptom of infection was the collapse of the tissue and the consequent reduction of the diameter of the infected portion. Its colour became light brown, gradually changing to dark brown, and finally to sooty black (Plate XXXIII, fig. 4). Numerous small blisters appeared on the surface, which later on burst into distinct longitudinal cracks. Innumerable conidia of *Coniothecium* could be seen with the lens all over the infected area.

The fungus was re-isolated in every case and resembled the parent culture in all respects.

Cultural studies

An extensive study of the behaviour of the fungus in oatmeal agar, Brown's starch synthetic agar and Czapek's agar was carried out. Cultures were made in Petri dishes of uniform size of 9 cm. diameter, each containing 10 c.c. of the medium. A small bit of hypha was planted in the centre of each dish. The inoculated dishes were kept in the room where the temperature varied from the maximum of 73°-87°F. to the minimum of 62°-71°F. The growth of the colonies was measured at intervals of four days up to 20 days and the macroscopic and microscopic characters were noted after a month. Conidia were never produced in these media.

Table I

Measurements of the linear growth of the colonies of Coniothecium chomatosporum *in three different media*

	Average diameter of the colonies				
	4th day	8th day	12th day	16th day	20th day
	mm.	mm.	mm.	mm.	mm.
Oatmeal agar	7·50	12·76	17·68	24·18	27·56
Czapek's agar	8·07	14·35	20·25	24·0	25·0
Brown's starch synthetic agar	7·20	19·91	18·75	25·5	29·0

In addition to these three media the fungus was also grown on potato-dextrose, Brown's synthetic, apple stem decoction and Coon's agar media and also on sterilised apple stems and leaves, to see if conidia formation could be induced. But they were not formed in these media also. In all

the media tried only the chlamydospores appeared (Plate XXXIII, fig. 4). The mycelium was in every case thick, light to dark brown in colour, highly granular and closely septate. There were faint and narrow zonations in the cultures.

The fungus was very slow growing in all the media. Its growth was inhibited at the temperature of 86°F. but it was resumed when the temperature was lowered.

Sources of infection

In New Zealand, where this fungus is known to attack apple stem and fruits, two spore-stages are found. The *Coniothecium* stage, which is parasitic, and a second stage, which is apparently saprophytic occurring on dead bark of old diseased areas. Until recently, only the *Coniothecium* stage was known. But Massee [1915] found a pycnidial or *Phoma* stage associated with *Coniothecium*, and he and Van der Bijl [1915], later on, were able definitely to link them together. In New Zealand the fungus overwinters on shoots either in the form of pycnidia, or in the tissues as mycelium of the *Coniothecium* stage. In Kumaun the pycnidial stage is not met with and the fungus overwinters in the mycelial form in the shoots of cankered branches. The primary conidia of *Coniothecium* seems to be the chief agent for the spread of the disease. The minute hyaline spores, produced by budding from the secondary spores originating from the primary conidia, may also serve as a source of infection.

The disease usually starts in July and the infection is the highest by the middle of August. Innumerable primary conidia and secondary spores were readily caught, towards the middle of August, on vaseline-smeared slides exposed in spore traps (aeroscopes). The disease can thus be held to be carried by wind. But it can also spread through rain water. Drops of water collected from cankered areas after a shower of rain showed numerous spores of the fungus in them. The pruned surfaces below a diseased area very frequently become infected, probably through contaminated drops of rain water. Whether they are carried by insects has not been determined.

Control

Since the mycelium remains imbedded in the tissue of the host, spraying or dusting will have no effect in controlling the disease. Butler [1919] observed that spraying with lime, sulphur and Burgundy mixture failed to check it completely. Careful pruning of the affected areas and branches appears to be the only means of getting rid of the disease where it is established. But to prevent fresh infection a paint or smear, which can well cover the cut surfaces of the branches and at the same time stop germination of the spores of the fungus settling on them, can be the only means.

With this point in view an experiment was laid out in 1936 to try the efficacy of pastes made with 100 c.c. raw linseed oil and each of the following ingredients and of Ostico banding grease :—

	oz.
Dried precipitate of Bordeaux mixture . .	4
Copper carbonate	4
Red lead powder	8
Red lead powder	2
Copper carbonate	2

The total number of treatments along with the controls in this experiment was seven, *viz.*, (1) untreated and uninoculated (control), (2) untreated and inoculated, (3) Bordeaux paste, (4) copper carbonate paste, (5) red lead paste, (6) red lead and copper carbonate paste and (7) " Ostico " banding grease. All these treatments were given on pruned surfaces on each of twelve old apple trees selected at random in different blocks of the orchard. Each treatment was replicated twelve times. The pastes were applied on the cut surfaces from the 14th to 18th July 1936, and barring the controls, all of them were inoculated with bits of the culture of *Coniothecium chomatosporum* from the 29th July to 2nd August 1936. This culture was growing on Brown's starch synthetic agar medium at room temperature for 15 days. After inoculation each surface was covered with a piece of sterilised wet cotton wool and enclosed in cellophane bags in order to maintain the moisture condition necessary for infection. The cellophane bags were removed after a fortnight. Infection was quite visible in every one of the untreated and inoculated surfaces and also in some of the treated ones. The experimental twigs were cut and brought into the laboratory for microscopic examination.

Table II gives the results of the analysis of variance and the comparisons of the mean percentage infection for the different treatments.

TABLE II

(*a*) *Analysis of variance*

——	Degree of freedom	Sum of squares	Mean square	" F "
Blocks	11	4941·7348	449·2486	7·70
Treatments	6	42517·6868	7086·2611	*121·43
Error	66	3851·5820	58·3570	

* Highly significant,

(*b*) *Summary of results*

——	Treatments							Standard error of difference
	1	2	3	4	5	6	7	
Mean percentage infection.	100	100	83	86	74	31	100	3·11

Conclusions :—(1) = (2) = (7) > (4) = (3) > (5) > 6.

It is clear from these tables that the paste made by mixing 2 oz. of red lead and 2 oz. of copper carbonate with 100 c.c. of raw linseed oil is found to be most effective in controlling fresh infection of the disease.

In 1937 the following treatments were, in addition, tried against painting with the paste made of red lead, copper carbonate and raw linseed oil to discover any other paste which would be equal to or better than the latter.—

(1) Painting with the paste prepared by mixing 2 oz. of red lead and 2 oz. of dried precipitate of Bordeaux mixture in 100 c.c. of raw linseed oil

(2) Washing the cut surfaces with 1-1000 $HgCl_2$ previous to painting with Bordeaux paste prepared by mixing 4 oz. of dried Bordeaux mixture with 100 c.c. of raw linseed oil

(3) Painting with a paste of linseed oil 100 c.c., and lime sulphur 4 oz. (equal quantities of lime and sulphur were self boiled before adding linseed oil).

The experiment was laid out in exactly the same way as that of 1936.

Table III gives the results of the analysis of variance and the comparisons of the mean percentage infections for the different treatments.

TABLE III

(*a*) *Analysis of variance*

——	Degree of freedom	Sum of squares	Means quare	"F"
Block	11	3221·093	292·826	2·28
Treatment	5	85760·7048	17132·1409	*133·51
Error	55	7057·6592	128·321	

*Highly significant

(b) Summary of results

	Treatments						
	Untreated (uninoculated)	Untreated (inoculated)	Red lead + copper carbonate paste	Red lead + Bordeaux paste	$HgCl_2$ followed by Bordeaux paste	Lime sulphur paste	Standard error of difference
	1	2	3	4	5	6	
Mean percentage infection.	100	100	8	32	66	84	4·6

Conclusions :—(1) = (2) > (6) > (5) > (4) > (3)

It is, therefore, proved that the paste prepared with equal quantities of red lead and copper carbonate in raw linseed oil was the best of all the pastes tried in preventing fresh infection by *Coniothecium chomatosporum*.

Summary

1. The stem-black disease of apple, caused by *Coniothecium chomatosporum* Corda, occurs extensively in every orchard in Kumaun.

2. The fungus infects pruned surfaces and proceeds downwards, rendering the affected areas jet black. Cankers are formed and the branches are ultimately killed. Thick branches are more susceptible than thin ones. The tissues are killed up to the cambium and the fungus hibernates in the tissues.

3. The conidia are dark brown, circiniform, muticate, often coalescent and are very variable in size. Some of the conidia burst and give rise to secondary hyaline spores. The secondary spores either grow into germ tubes or produce buds. The buds on their part also grow into germ tubes.

4. The spores are carried by wind and with rain water.

5. The fungus cannot enter through uninjured surface of the stem but can do so only through wounds.

6. A detailed cultural study of the fungus is made. The fungus is slow growing and does not produce conidia in any of the media tried. Only chlamydospores are formed.

7. Painting of the pruned surfaces with a paste made of 2 oz. each of red lead and copper carbonate and 100 c.c. of raw linseed oil very effectively controlled the spread of the disease.

ACKNOWLEDGMENT

The writers are deeply indebted to the Imperial Council of Agricultural Research, for the grant of funds for the Hill Fruit Research Scheme of the United Provinces, with the help of which the work was carried out at the Government Fruit Research Station, Chaubattia, United Provinces. They are also grateful to the Statistician of the Council for scrutinising Tables II and III and re-arranging them in their present form.

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A FRUIT ROT OF APPLES CAUSED BY A SPECIES OF *RHIZOPUS*

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(With Plate XXXIV and two text-figures)

INTRODUCTION

VERY few fungi associated with " rots " of ripe apple fruits are recorded in India and probably none on unripe fruits. In the United Provinces [Dey, 1933] it has been found that *Aspergillus niger* Van. Tieghem is the common organism causing soft rot of apple. In other countries, on the other hand, many fungi are known to damage apples in storage [Rose, 1924].

In the course of investigations on codling moth of apples in Quetta, the samples sent to the Imperial Entomologist were often received in a rotting condition. In many cases such fruits were also observed on the trees. These samples were sent throughout the months of June and July 1937 and consisted of fruits in different stages of maturity, but usually they were unripe. In most cases (over a hundred fruits examined) the freshly rotted portions gave a pure culture of a species of *Rhizopus*. Some fruits, especially those approaching maturity, showed *Aspergillus niger* on the rotted portion. It was rather noticeable that in such fruits a species of *Rhizopus* did not occur although the samples of fruits rotted by the two fungi were packed in the same lot.

Preliminary experiments showed that the species of *Rhizopus* was a vigorous wound parasite, capable of attacking the ripe and green apples alike*. It has not yet been possible to ascertain the amount of damage due to this fungus but on two occasions rotted apples purchased early in the season (August) from the local market revealed the presence of a similar species of *Rhizopus*.

In 1938 a further sample was received, this time from Kurram Valley. There was no sign of insect attack. In a letter sent by the Extra Assistant Director of Agriculture, Kurram Valley, it was stated that the apples first changed from green to yellow and then pink before becoming brown. Rotting was confined to the stem-end, and was found to be caused by a species of *Rhizopus* similar to that found in the previous year.

*A preliminary account of this work has appeared in *Current Science*, 6, 58-9, 1937.

SYMPTOMS, MORBID ANATOMY AND MORPHOLOGY

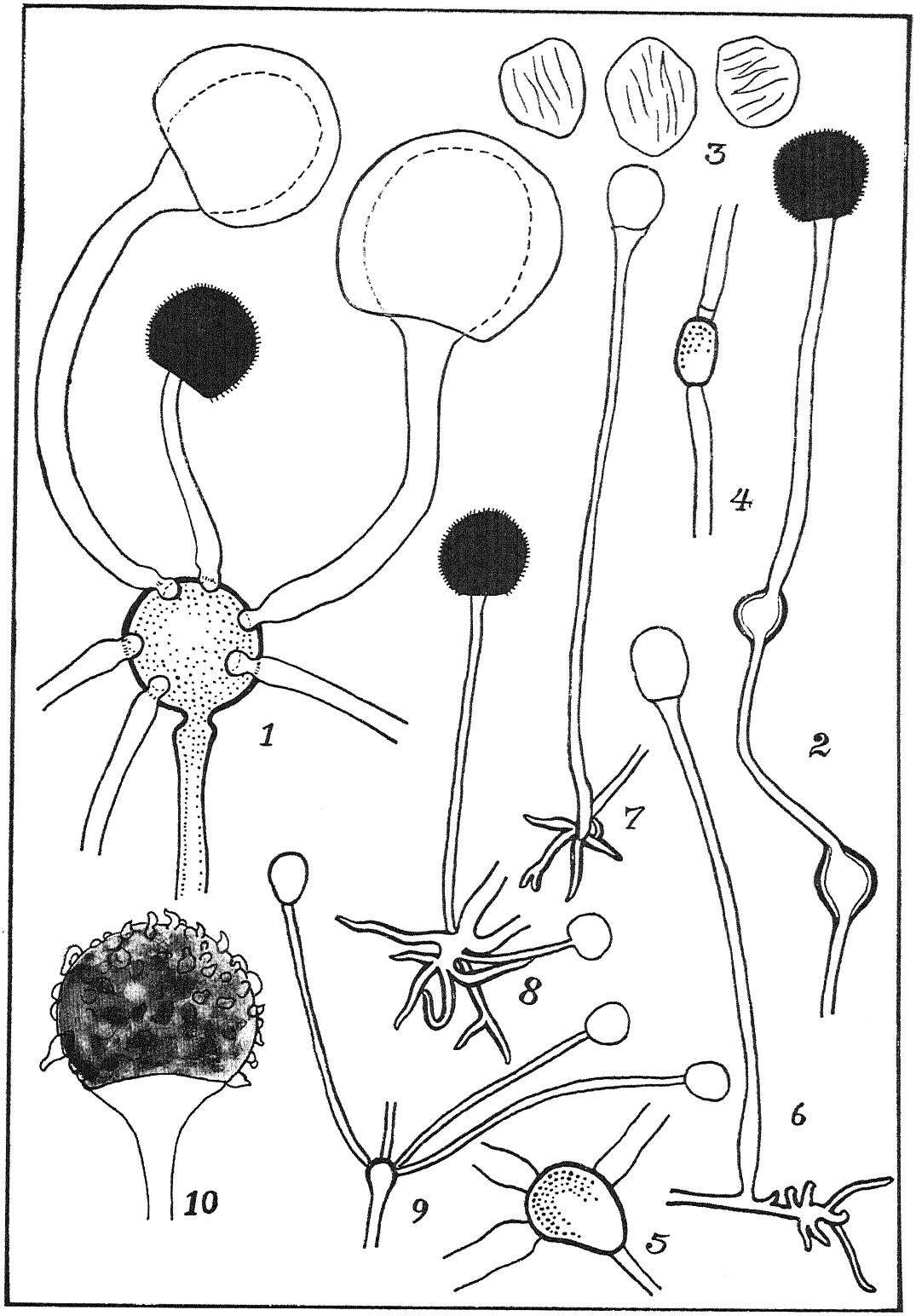
Symptoms.—The rotting fruits in the earlier stages did not show any exudation of liquid. In the advanced stages of decay the skin on the whole surface was thrown into wrinkles and a liquid exuded. There was no progressive sinking of the originally infected patch nor was the surface moist and shiny as noted by Dey [1933] in the fruits affected by *A. niger*. On the " Kulu " variety of green apples (flat, yellow-skinned fruits) the skin of the affected portions became " russet " coloured or " verona-brown " [Ridgway, 1912] and could be easily peeled away from the underlying tissue. The latter was discoloured to zinc-orange. The fruits had a slightly sour but not unpleasant odour. Since the core hardly offered any resistance, they were easy to cut across. The scales within which the seeds were enclosed easily became detached from the tissue of the core.

There was no fungus growth on the surface of the fruits but on cutting them open it was noticed that the seeds were covered with a weft of mycelium studded with black bodies. In certain cases the seeds were absent but a wad of mycelium was present in that location. Whenever the skin was removed or the fruit cut open, whitish aerial mycelium appeared on the surface within 24 hours.

Morbid anatomy.—Microscopic examination of the pulp showed the presence of broad (12-16 μ), coarse, aseptate light brown hyphae following a tortuous course, probably due to their being intercellular. These main hyphae produced numerous short, narrow, lateral branches which usually penetrated the host cells and sometimes the intercellular spaces also. The host cells abutting on the main hyphae were darker brown than those further away and these hyphae could, therefore, be easily traced. In the green apples there was no killing in advance ; the rotting was closely associated with the presence of hyphae.

Morphology.—The morphology of the fungus on the host and on culture medium was studied. The fungus was isolated from the diseased tissues and from the fungus growth on the seeds. The macroscopic and microscopic growth characters of the different isolations were compared on agar medium and found to be identical. Eventually single spore isolations from these cultures were made.

The mycelium occurring on the seed and within the scales containing the seeds was composed of broad (12-20μ) aseptate hyphae with a few isolated, intercalary hyaline chlamydospores (20 × 14μ). Rhizoids were rare. The sporangiophores were borne on bulbous brownish swellings 40-80μ in diameter of the hyphae (Plate XXXIV, figs. 1 and 2), or were terminations of ordinary hyphae. On these bulbous swellings one to many brownish sporangiophores (usually 1 to 3) were produced in the manner figured for *R. arrhizus* Fischer [Zycha, 1935] and for *R. nodosus* Nym. [Lendner, 1908]. These sporangiophores measured 160-480 μ in length and 10-12 μ in width. The sporangia were dark, more or less globose, (Plate XXXIV, fig. 1), 80-176 μ in diameter



1. A group of sporangiophores arising from the brown bulbous swelling. Taken from host (× 300). 2. One sporangiophore and two bulbous swellings on the hyphae. Taken from host (× 160). 3. Spores (× 1800). 4. Intercalary chlamydospore (× 300). 5. Intercalary chlamydospore (× 300). 6—9. Different sizes and manners in which sporangia are produced on malt agar (× 160). 10. A deformed sporangium. Taken from host (× 300).

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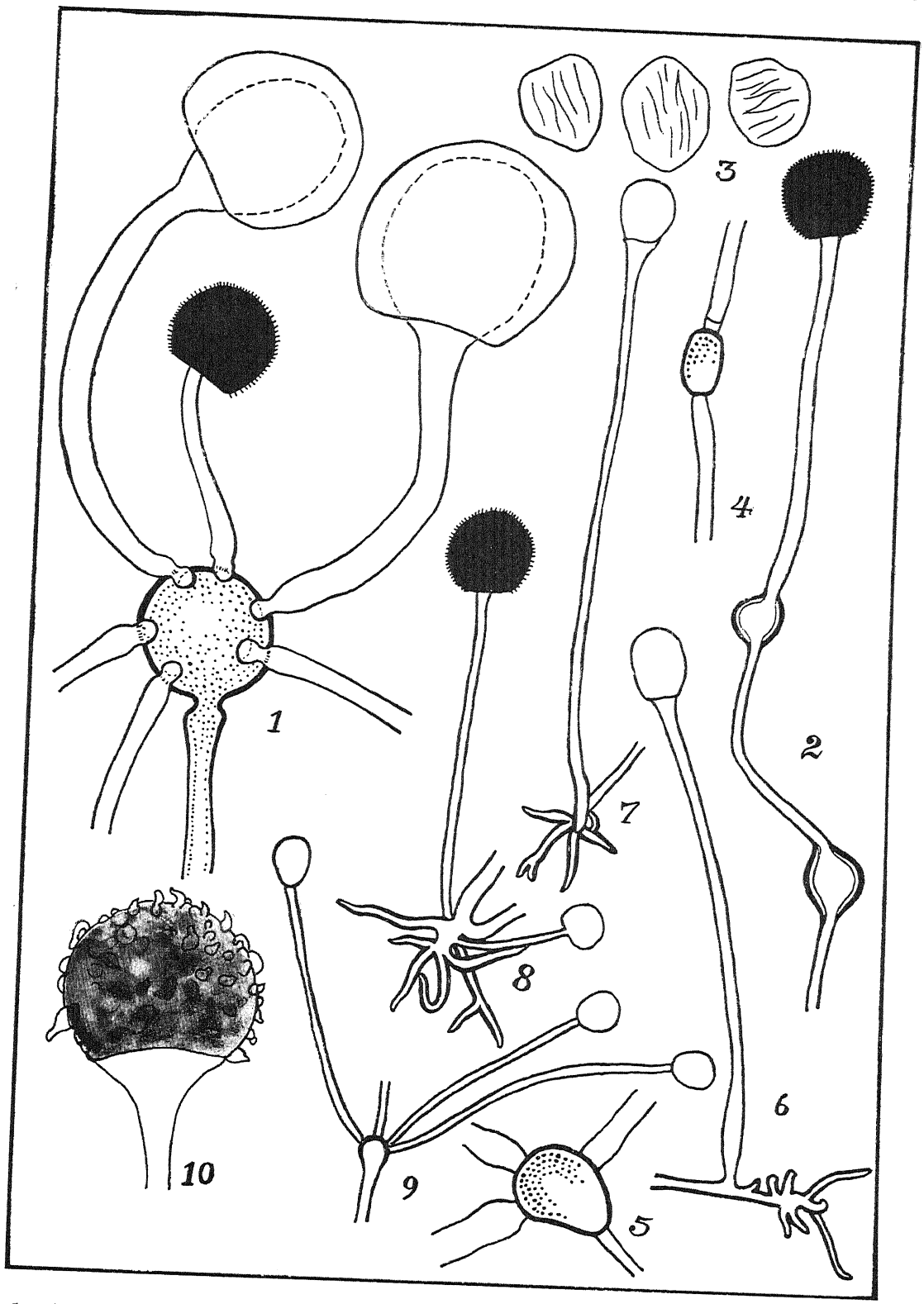

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mean 114 μ (100 measurements). The columella was more or less oval, 60-100 × 66-112 μ with a mean diameter of 88 × 96 μ. A small percentage of deformed sporangia were also sometimes noted. In these the contents instead of differentiating into spores developed into numerous small, hyaline chlamydospore-like bodies with a very characteristic shape (Plate XXXIV, fig. 10). Such sporangia have been noted previously [Yamamoto, 1930] to occur commonly in the *Rhizopus albus* group.

The spores were light grey, plain-surfaced, slightly angular (nearly polyhedral), faintly striated and measured 5-7·2 μ, with a mean of 7·0 μ (200 measurements).

Good aerial growth, which was first white but later light brownish, occurred on oat agar, malt agar (3 per cent), potato-dextrose agar, and apple extract agar, but it was poor on Hopkins' and Dox's agar. On these media light to dark brown rhizoids developed but their formation was poor on Hopkins', Dox's and apple extract agar. Their formation was not correlated with the amount of aerial growth, for on apple extract agar medium and cut apples aerial growth was copious but rhizoids were scanty. The stolons were hyaline except for the small length near the rhizoids. Hyaline chlamydospores developed in stolons as well as in the ordinary hyphae. They were formed close to a septum (Plate XXXIV, fig. 4).

Sporangiophores and sporangia were copiously formed on most of the media. The sporangiophores arose on the nodes (rhizoids), on the stolons and from the ordinary hyphae (Plate XXXIV, figs., 6-9).

In cultures a great variation in the size and shape of the sporangia, depending on their position, was noted. Those which were produced on the stolons and nodes conformed to the size and description given previously ; those which developed on the aerial hyphae, on the other hand, were often much smaller, more flat (sub-hemispherical) and their columella was ellipsoidal. The bulbous swellings on hyphae, mentioned previously, were rather infrequent in cultures, and when they did appear it was more commonly in culture tubes than in Petri-dish cultures.

Abnormal sporangia were rare on some media and very frequent on others. Yamamoto [1930] has emphasised this character in the key for the classification of the species of *Rhizopus*. The studies made on this particular fungus, suggest that this is not a very stable character. These abnormal sporangia were common on apple extract agar (*p*H 5·2). Their contents broke up, sometimes, into a very few big segments (4 to 8), but more commonly, however, into numerous hyaline beaked or curved bodies. Sometimes a part of the protoplasm of the sporangium developed normal spores and the rest into the above mentioned bodies. Direct germination of the sporangium was also noted but very rarely. These abnormal sporangia tended to be more flat and brownish.

The size and shape of the sporangium did not seem to be affected by temperature. The striations on the spores, however, looked more prominent at 26°C, than at 15° or 32°C.

Infection experiments

Preliminary infection experiments were made on a few varieties of ripe apples available in the local market. Fruits were surface sterilised and sporulating mycelium was placed on the surface which was then gently pricked with a sterile needle to injure the skin. These apples were placed in sterile, moist chambers at the room temperature (96-100°F). Definite spots were found (0·5 to 2 cm. in diameter) in all the inoculated apples within 24 hours while the controls remained unaffected. Out of 21 inoculations 20 were successful. The size of the spot varied from apple to apple, even on the same variety. In the purple-skinned varieties there was an intensification of the colour at the margin of the spot. The depth of rotting increased at a rate equal to the increase in radius of the spot at the surface. Examination of completely decayed apples showed that the fungus had enveloped the seed with a weft of mycelium just as in a naturally infected fruit.

In order to carry out the infection experiments critically, semi-mature green-skinned fruits (slightly sweet to the taste) of one variety and age were obtained from Quetta. The method of inoculation used was that of Granger and Horne [1924]. All the apples used in the experiment were first placed for one day at 35°C. to see that they carried no previous infection. Each apple was then weighed. With a sterile cork borer a cylinder of tissue was removed to a depth of half an inch. A thick spore suspension (about 0·1 c.c.) was introduced with a sterilised pipette, the plug was replaced, and the wound was sealed with paraffin. The apple was wrapped in tissue paper and placed at the required temperature. At the expiration of the desired time it was removed, the rotten tissue was scraped away, and the healthy tissue was weighed. The experiment was conducted in triplicate at temperatures between 32°C. and 40°C. and in duplicate for lower temperatures. The effect of temperature on the percentage of rotting of green fruits is shown in Table I.

Table I

Effect of temperature on the percentage apple tissue rotted

Period of incubation (hours)	Percentage of rotting				
	24°C.	32°C.	35°C.	37°C.	40°C.
24	0·8	3·6	5·2	7·0	8·5
48	2·2	14·2	19·6	26·5	20·1
72	6·8	56·0	81·4	100·0	100·0
96	12·6	94·4	100·0	..	..
120	22·0	100·0	..	..	..
144	31·5	..	..	..	..

It will be seen from Table I that rotting occurred from 22°C. upwards and its rate was very fast from 32°C. upwards.

Effect of temperature on growth of the fungus

Experiments were also made to determine the thermal relations of the fungus on agar media. Plates of equal sizes uniformly poured were used in triplicate. Dox's, Hopkins' and malt agar media were used but, as the former two media gave very sparse, fluffy growth, they were found unsatisfactory. The fungus was very fast growing and in forty-eight hours at 35° and 37°C. it filled Petri-dishes of malt agar 11 cms. in diameter.

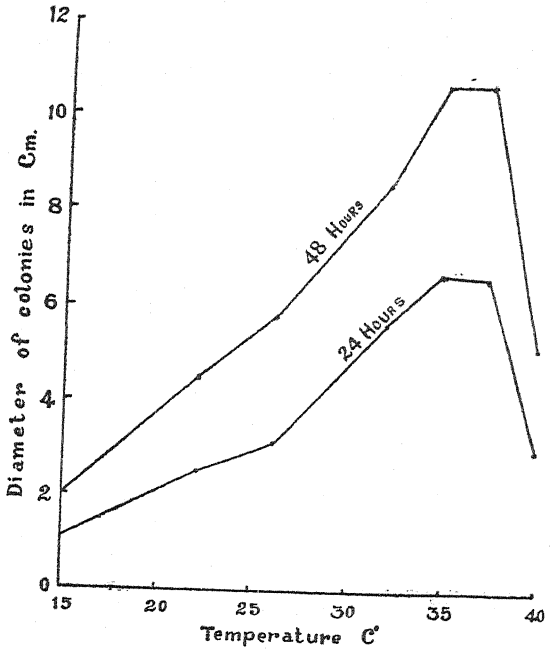

Fig. 1. Rate of growth of *Rhizopus* from apple on malt agar at different temperatures.

It can be seen from Fig. 1 that the fungus could grow from 15° to 40°C. and the optimum temperature for its growth was between 35° and 37°C. These results are in conformity with those obtained in infection experiments. It is, however, not clear why inoculated fruits were affected as seriously at 40°C. as at 35° to 37°C. Either it is due to the adverse effect of the temperature on the fruits, or to the fact that their internal temperature was lower and thus corresponded with the optimum requirements of the fungus.

Effect of reaction of medium on growth of the fungus

As stated previously, the fungus could infect both ripe and green apples which were presumbaly of different acidities. The effect of hydrogen-ion concentration on the growth of the fungus was therefore studied. Peptone

solution (2 per cent) was adjusted to different *p*H by adding varying amounts of acid and alkali according to the method of Karrer and Webb [1920]. The solutions were then inoculated by adding equal amounts of a spore suspension. After 24 hours a visible sign of growth was present in solutions in the range of *p*H from 3·0-8·4. At *p*H 3·0 and 3·2 there was a little gelatinous, submerged growth which tended to become aerial with age ; at *p*H 4·1-8·4 aerial growth was good, being most copious at *p*H 4·1 and 4·4 and decreasing progressively in more alkaline ranges. Sporangia developed from *p*H 4·1 to 8·4 but were more abundantly formed in acidic media. The fungus changed the sulphur yellow colour of the medium to orange-cinnamon at *p*H 8·4.

The dry weight of the fungus formed at different hydrogen-ion concentrations is shown in Fig. 2.

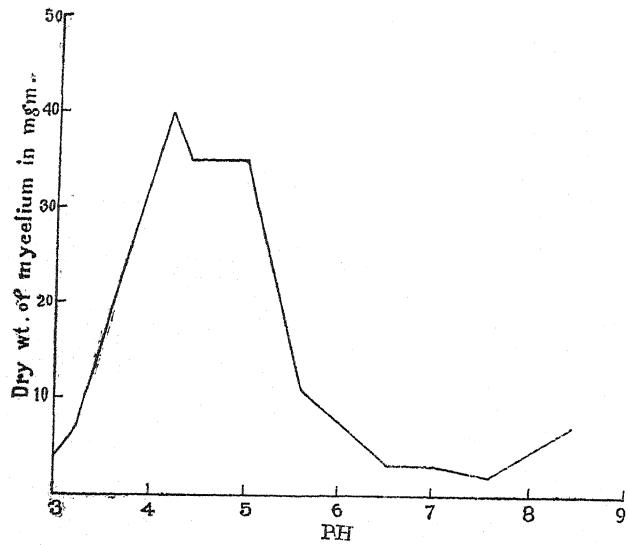

FIG. 2. Effect of hydrogen-ion concentration on the growth of *Rhizopus* from apple (after fourteen days)

The fungus tended to make the medium alkaline, a tendency especially marked in acid range. In the alkaline range it tended to be slightly more alkaline except at *p*H 8·4 where the medium became slightly acidic (*p*H 8·1).

The *p*H of the green apple was found to be 4·1 to 4·2 and the ripe apples (used in inoculation experiment) 5·2. Both these fruits, therefore, afforded a suitable substratum for the growth of the fungus, specially the green fruits. The fungus changed the *p*H of the latter from 4·1 to 5·2.

IDENTITY OF THE FUNGUS

The formation of bulbous swellings from which the sporangiophores arise has been emphasised by Lendner [1909] as a specific character of *R. nodosus*. Somewhat similar swellings were noted by Hanzawa [1912] on *R. Delemar*

(Boid.) Wehm. et Hanz. The presence of sporangiophores on swollen nodes, the scarcity of rhizoids, and the size of the spores suggest that the species under consideration is *R. arrhizus*. Zycha [1935], however, suggests that there is no valid ground for the separation of *R. arrhizus* and *R. nodosus*. He also states that both Lendner and Hagem hesitated in separating them. Yamamoto [1930,1], however, does not include *R. nodosus* in the " arrhizus group " but places in ' Oryzae group ' where the sporangia and rhizoid formation is very good and the stolons are coloured. He also separates the two species on the basis of physiological characteristics [1930,2]. He found that *R. arrhizus* formed sporangia at 15°C., but not at 41°C. while *R. nodosus* did so at both the temperatures. In the present studies sporangia developed at 15°C. and not at 41°C, while the stolons were either hyaline or pigmented to a variable distance from their point of origin on the nodes. The development of rhizoids was poor on certain media while good on others.

The species under study is assigned to *R. arrhizus* Fischer with the reservation as to its validity made by Zycha [1935] and as understood by him.

Conclusion

The parasitic potentialities of the group of the species *Rhizopus*, which are high temperature fungi, is well known in other countries and their parasitism has been established by Harter and Weimer [1922]. They state that many of these species of " intermediate temperature group " were vigorous parasites but in the United States of America none of these was met with as frequently in nature as *R. nigricans*. The presence in India of a fungus which thrives well at high temperature is not surprising.

The extent of damage to apples due to *R. arrhizus* is not yet known.

Summary

1. In the course of an investigation on the codling moth of apples in Quetta, the samples sent to the Imperial Entomologist were often received in a rotting condition. The fruits were partially ripe and had very characteristic symptoms. A further sample of fruits showing similar symptoms was received from the Kurrum Valley. Here the rotting started at the stem end while the fruits were borne on the trees.

2. The affected fruits turned yellowish brown and remained quite firm in the earlier stages of the disease. These fruits had a slightly sour but not unpleasant odour. When the fruits were cut open the seeds were invariably found embedded in a white pad of fungus mycelium. In the tissues the fungus tended to be intercellular and sent numerous short lateral branches into the cells.

3. The causal organism was identified as a species of *Rhizopus*. The mycelium occurring on the seed was composed of broad aseptate hyphae, and the rhizoids were rare. The sporangia were produced on bulbous brown swellings (1 to 3 or more on each swelling). The spores were 5-7·2 μ in diameter. Intercalary chlamydospores were present.

4. Infection experiments on ripe and unripe apples gave a very high percentage of infection. Slight wounding was necessary to start the infection. Severity of rotting was dependent on the temperature ; it was slow from 15-24°C. and very fast from 32-40°C. At the latter temperatures complete rotting occurred after 96 hours. There was some correlation between the thermal relation of the fungus in culture medium and on the fruits as evidenced by the rate of rotting.

5. The fungus tolerated a wide range of *p*H from 3·0 to 8·4, but thrived best at 4·2 to 5·0. The *p*H of green apple was found to be 4·2 and the ripe apple 5·2. Both these, therefore, afforded a suitable substratum for growth of the fungus.

6. The identity of the fungus is discussed and the species under study is assigned to *Rhizopus arrhizus* Fischer with the reservation as to its validity made by Zycha [1935] and as understood by him.

ACKNOWLEDGMENT

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A NOTE ON BOTRYTIS-ROT OF GRAPES IN THE QUETTA VALLEY

BY

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(With Plates XXXV—XXXVII and one text-figure)

INTRODUCTION

Botrytis-ROT of grapes has been reported in Europe by Muller, Viala [1891], Ravaz, Cuboni and Berro Aguilera [1926], in America by Rose and Ballard [1927] and Perold [1927], and in South Africa by Deyer [1931], and Du Plessis [1934]. In India, it has been recorded for the first time by the author in the Quetta Valley. Two species of *Botrytis, viz.*, *Botrytis cinerea* Pers. and *Botrytis vulgaris* Fr., have been found responsible for the disease. The latter is reckoned by many workers as a sub-species of *Botrytis cinerea*. In Southern Europe, where *Sclerotinia fuckeliana* De Bary is found associated with *Botrytis cinerea*, and is reckoned to be the perfect stage of this fungus; the former name is in common use.

The disease is named gray rot, noble rot, porriture grise, porriture noble, granfaule, edelfaule, vaalvrot and *Botrytis*-rot, etc. The author prefers the name *Botrytis*-rot to gray rot as strictly speaking the colour of the mould is not gray but grayish olivaceous. The fungus conforms with *Botrytis vulgaris* Fr., described by Saccardo [1886]. No ascigerous stage has been observed in culture or in the fields associated with this fungus.

DESCRIPTION OF THE DISEASE

Botrytis-rot of grapes is characterised by the decay and rot of berries associated with a grayish olivaceous growth of the fungus. The affected berries turn soft and brown and generate a pleasant smell. Infection usually starts from a cracked berry and spreads from it to other parts of the bunch. Sometimes the berries may crack before rotting. The fungus has been found to be capable of attacking healthy berries, though bruises and cracks facilitate its spread. Under humid conditions, the disease is fairly destructive and associated with powdery mildew of grapes takes a heavy toll of the crop.

DESCRIPTION OF THE FUNGUS

Botrytis vulgaris Fr. Syst. Mycol. III, page 398, Sacc. F. Ital. T. 693, *Polyactis vulgaris* Link., Corda Ic. I. Fig. 250, *Botrytis polyactis* Link., *Monilia vulgaris*, Pers. Sacc. Syll. Fung. Vol. IV, Pp. 128-129.

Clusters grayish olive, hyphae in cottony masses, whitish olivaceous when young, becoming olivaceous with age, ascending to erect, septate, branching above; branches short, spready, secondary branches few, usually opposite; conidiophores large, erect, olivaceous, constricted, several millimeters high, 10-25 μ. thick, simple or branched; secondary branches 7-9 μ. thick near the head, gradually attenuated to 3-4 μ. at the ends. Heads of conidiophores sub-hyaline, granular, smooth or slightly verrucose. Conidia oval, oblong-oval or globose, 6-9 by 7-13 μ., 7 by 10 μ. predominant, granular, sub-hyaline appear grayish olivaceous in clusters, acrogenus or acropleurogenus.

Conidia germinate readily in distilled water and 0·1 per cent glucose solution at 30°C., one to three germ tubes having been observed (Plate XXXVI, fig. 3). Conidial formation is more abundant in light than in darkness. Infection, for the most part, takes place by the spread of these conidia. Germ tubes are capable of penetrating through healthy skin, the degree of penetration varying with the ripeness of the berries. Hyphae invade all parts of the berry, growing within the cells as well as between them, and ramifies through the tissue of grapes surrounding and filling them with mycelium. As a result of infection the cells are destroyed, the protoplasm shrinks away from the cell's wall and becomes disorganised.

Sclerotia.—Sclerotia are abundantly formed in culture (Plate XXXVI, fig. 1). In older growth certain hyphae twist round to form concentric coils of hyphæ which generally become thick to form minute knot-like bodies. These are the young sclerotia which are at first whitish in colour, but later develop a dirty greenish tinge. As they advance in age they turn dark brown and finally become almost black. Mature sclerotia (Plate XXXV, fig. 10-10c) are dark fuscus or black, 3-10 mm. in diameter, irregular in shape, hairy; hairs thick, black, coriaceous; wall of the sclerotium dark brown, of more or less polygonal cells, and under it is a hypodermal layer of dark brown cells and a central medullary zone of compact, thick, hyaline and granular hyphæ. Wefts of hyaline hyphæ connect the sclerotium with the mother mycelium and serve as feeders, when the sclerotia are young; the hairs seem to be mainly protective.

Secondary conidiophores.—After a period of rest which ranges from a few days to several weeks, the sclerotia form all over their surface conidiophores of the normal type (Plate XXXV, fig. 10-c) The vast majority of these conidiophores bear conidia in acropleurogenus manner. The conidia from these germinate and form new mycelia which in turn repeat the life-cycle. Conidial formation is more rapid in light than in darkness.

Microconidia.—Occasionally microconidia are formed. Certain conidia on germination form two germ-tubes, one of which develops into a haustorial filament, while the other forms a short fairly branched conidiophore which bears microconidia in chains (Plate XXXVI, fig. 2). These microconidia do not seem to possess any power of germination and are not of much consequence in the life-cycle of the fungus.

PLATE XXXV

FIG. 1. Conidiophore of *Botrytis cinerea* with terminal clu-ter of acrogenous conidia, (× 100) i.e. part of conidiophore with acropleurogenous arrangement of conidia (×100)

FIG. 2. Head of conidiophore (× 629)

FIGS. 3a–3e Showing different modes of branching of the head of the conidiophore (× 100)

FIGS. 4–6. Heads of certain types of conidiophores magnified (× 900)

FIG. 7. A secondary branch of the head of young conidiophore, bearing young conidia (× 900)

FIG. 8. Conidia (× 629)

FIG. 9. Germinating conidia with 1–3 germ tubes in different stages of development (× 629)

FIGS. 10–10c Sclerotia in different stages of development, 10a Young sclerotium in culture (nat. size), 10b–10f Mature sclerotia (×3), 10c Sclerotia with secondary conidiophores (×3)

FIG. 11. Section of the sclerotium showing sterile hairs coniceons wall, hypodermal layer of dark brown cells shown light dark and central medullary zone of hyaline or sub-hyaline hyphae. These single threads shown as hairs represent sub-hyaline webs of hyphae which are primary source of nutrient supply to sclerotium (× 100)

FIG. 12. Sterile hairs of sclerotium (× 100)

FIG. 13. Showing texture of the wall of the sclerotium (×900)

FIG. 14. Part of medullary zone of the sclerotium (×900)

PLATE XXXV

FIG. 1. Conidiophore of *Botrytis vulgaris* with terminal cluster of acrogenus conidia, (× 100) 1 *a*. part of conidiophore with acropleurogenus arrangement of conidia (× 100)

FIG. 2. Head of conidiophore (× 625)

FIGS. 3—3*a* Showing different modes of branching of the head of the conidiophore (× 100)

FIGS. 4—6. Heads of certain types of conidiophores magnified (× 960)

FIG. 7. A secondary branch of the head of young conidiophore, bearing young conidia (× 960)

FIG. 8. Conidia (× 625)

FIG. 9. Germinating conidia with 1-3 germ tubes in different stages of development (× 625)

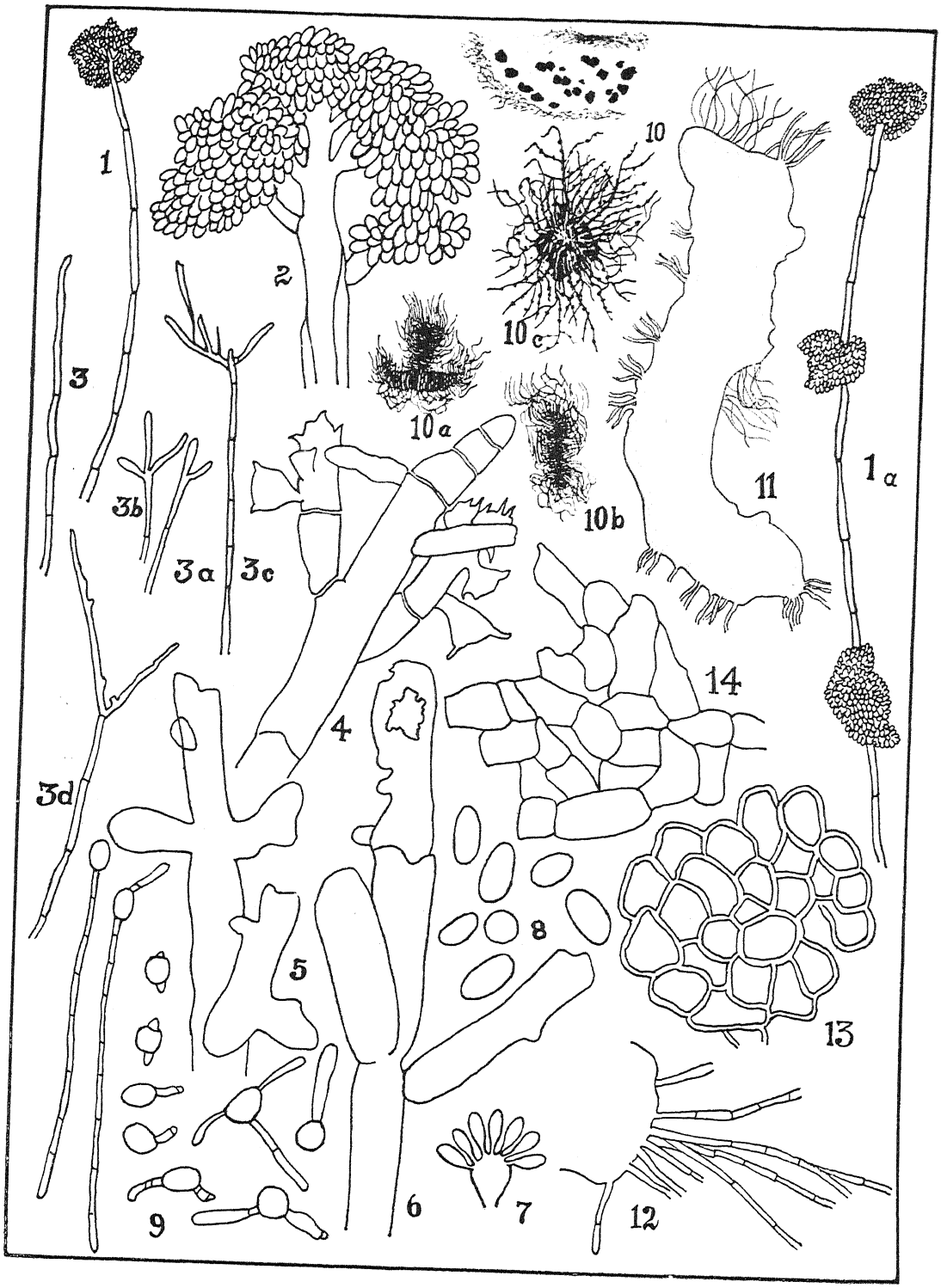
FIGS. 10–10*c* Sclerotia in different stages of development, 10*a* Young sclerotium in culture (nat. size), 10*a*–10*b* Mature sclerotia (×3), 10*c* Schlerotia with secondary conidiophores (×3)

FIG. 11. Section of the sclerotium showing sterile hairs coriaceous wall, hypodermal layer of dark brown cells shown light dark and central medullary zone of hyaline or sub-hyaline hyphae. These single threads shown as hairs represent sub-hyaline wefts of hyphae which are primary source of nutrient supply to sclerotium (× 100)

FIG. 12. Sterile hairs of sclerotium (× 100)

FIG. 13. Showing texture of the wall of the sclerotium (×960)

FIG. 14. Part of medullary zone of the sclerotium (×960)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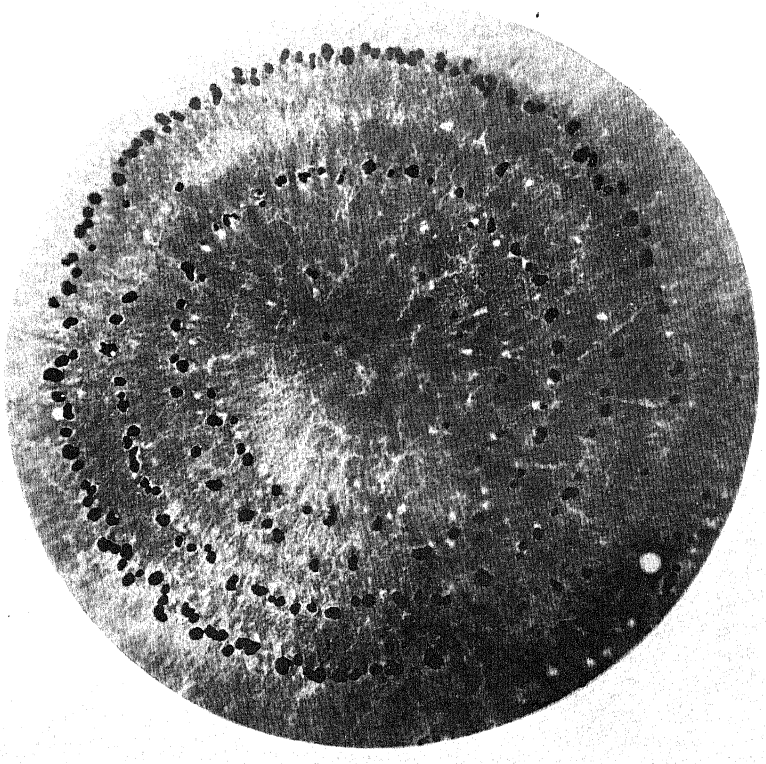

FIG. 1 Photograph of colony of *Botrytis vulgaris* Fr. on oat agar, incubated at 30°C. for 7 days, showing the formation of sclerotia in concentric rings

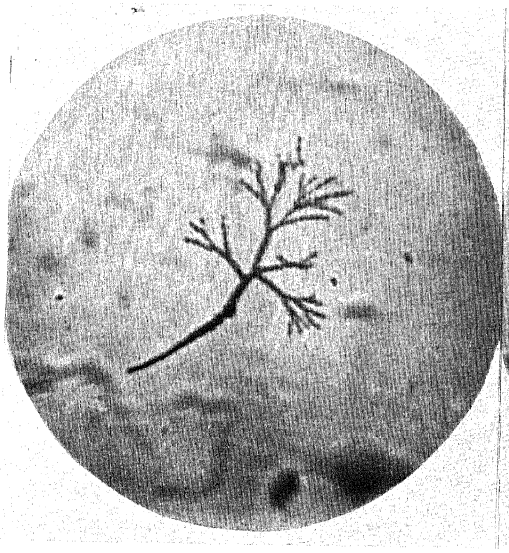

FIG. 2 Photomicrograph of a microconidiophore formed by the germination of a spore of *Botrytis vulgaris*. Microconidia are borne in chains

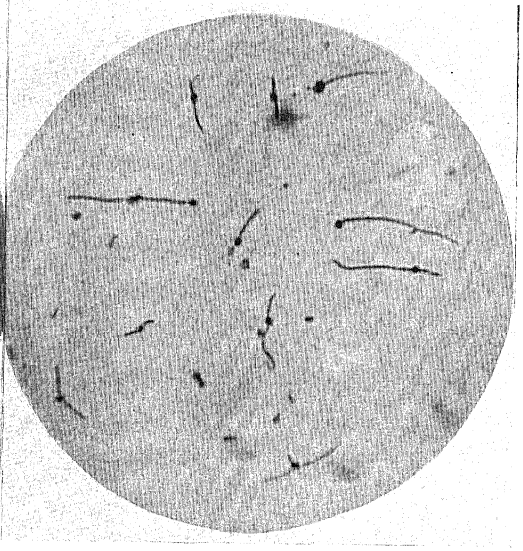

FIG. 3 Photomicrograph of germinating spores of *Botrytis vulgaris* ; 1 to 3 germ tubes are shown

FIG. 1 Photograph of a small bunch of grapes kept as control during inoculation experiments.

FIG. 2 Photograph of a small bunch of grapes artificially infected with spores of *Botrytis vulgaris* Fr. (after 5 days).

FIG. 3 Photograph of a small bunch of grapes artificially infected with spores of *Botrytis vulgaris* Fr. (after 10 days).

Anastomosis.—Anastomosis or hyphal fusions have been often observed between two germ-tubes or between hyphæ and germ-tubes, affording a possibility for the interchange of cell contents. This was first noted by De Bary and has been observed by many others. Recently Hansen and Smith [1932] have reported the occurrence of microconidia and anastomosis. The frequent occurrence of multinuclear condition in hyphal cells and spores of *Botrytis* and such other fungi has suggested to these workers the possibility of irregular distribution of genetically dissimilar nuclei in cell-division or conidial formation. This phenomenon has been the basis of considerable speculation in relation to the origin of variation in fungi.

Ascigerous stage.—No ascigerous stage has been found in culture or in the fields associated with this fungus. All attempts to stimulate sclerotia to form apothecia failed. The sclerotia were isolated from cultures and thoroughly washed with sterilised distilled water. These were then placed in dishes on moist sand, quartz and filter paper and kept at 7°C. in darkness for a period of two months, but no apothecial fundaments were observed; conidiophores, however, were formed in abundance. The sclerotia were kept under further observation for six months but no apothecia appeared.

Pathogenecity

Inoculation experiments were organised to study the pathogenecity of *Botrytis vulgaris* Fr. Small bunches of grapes were taken. The berries of four bunches were pricked with a sterilised needle. Two of these were washed with the suspension of *Botrytis* spores in sterilised distilled water and the other two were washed with uninfected sterilised distilled water; each bunch was kept in a pair of Koch's culture dishes containing small amount of sterilised distilled water to maintain humidity. Dishes were kept at the room temperature. Four more bunches of grapes were taken. All these were kept unbruised. Two of these were washed with suspension of *Botrytis* spores in sterilised water. Each bunch was kept in a pair of Koch's culture dishes as described above. Within 24 to 48 hours the infection took place in all the cases where the bunches were washed with suspension of spores, while the others remained unaffected. Within a week the infected grapes were badly infested with the fungus (Plate XXXVII, fig. 2) and showed signs of decay while the controls remained almost healthy. The infection in case of berries pricked with needles was very rapid as compared to those unbruised. The fungus is capable of piercing healthy cell-walls. The experiment was repeated and identical results were obtained.

Physiology of the fungus

Prune agar, standard synthetic agar, potato-dextrose-peptone agar, oat agar, Richard's media and Coon's agar were the media used for the cultivation of this fungus. The formulæ used in the preparation of these media were those recommended by Rawlins [1934]. Petri dishes of uniform size (9 cm. in diameter) were used and 20 cc. of media was poured in each dish to have a

uniform thickness. Six plates of each medium were poured and a small piece of mycelium bearing conidia was planted in the centre of each dish and plates were incubated at 30°C. The growth character and the spread of the fungus after incubation for one week is presented in Table I.

TABLE I

Growth character and spread of Botrytis vulgaris *Fr. in different media, at 30°C.*

Media	Growth character	Average spread of colony in 7 days (Diameter in mm.)
Prune agar . . .	Comparatively poor growth, sterile mycelia abundant, few or no sclerotia, sporulation poor	28·3
Standard synthetic agar .	No sclerotia, thin growth, conidiophores fairly common	26·7
Potato-dextrose-peptone agar	Thick, good flaky growth, vegetative growth predominant. Mycelia dirty white, no sclerotia, conidiophores comparatively poor	26·2
Oat agar	Sporulation, vegetative growth and sclerotial formation abundant	33·7
Coon's agar . . .	Sporulation, vegetative growth and sclerotial formation fairly good	27·1

The effect of different temperatures on the growth of *Botrytis vulgaris* was also studied. Oat agar was the medium used; 36 plates were poured; each dish was of uniform size, containing 20 cc. of media. These were inoculated with the fungus and sets of six such plates were incubated at each temperature. Growth at six different temperatures, *viz.*, 7°C., 19°C., 23°C., 27°C., 30°C., and 37°C., was studied. Sporulation was most active at 7°C., it gradually diminished with advance in temperature. At 27°C., there was again intense sporulation; then there was sudden fall and it came almost to a standstill at 37°C. Sclerotial formation was fairly active at 7°C. With the advance in temperature it increased and was at its highest at 27°C. Then it began to decrease and there was slight sclerotial formation at 37°C. As regards aerial mycelium, 23°C. was found to be the optimum temperature for its growth and at 37°C., mycelial growth was more or less completely inhibited. The effect of different temperatures on the growth of this fungus is shown in Table II and is graphically represented in Fig. 1.

TABLE II

Effect of different temperatures on the growth of Botrytis vulgaris *Fr.*

No.	Temperature	Aerial mycelium*	Sporulation*	Sclerotial formation*	Spread of colony in 7 days (diameter in mm.)
1	7°C.	+	++++	+++	38·8
2	19°C.	++++	+++	++++++	46·7
3	23°C.	++++++	++	+++++++	78·5
4	27°C.	+++	++++	++++++++	27·3
5	30°C.	+	++	++++	26·6
6	37°C.	—	—	++	9·6

*—denotes absence or negligible presence.
+, ++ denote slight and moderate growth respectively.
+++ to +++++++ show varying density of occurrence.

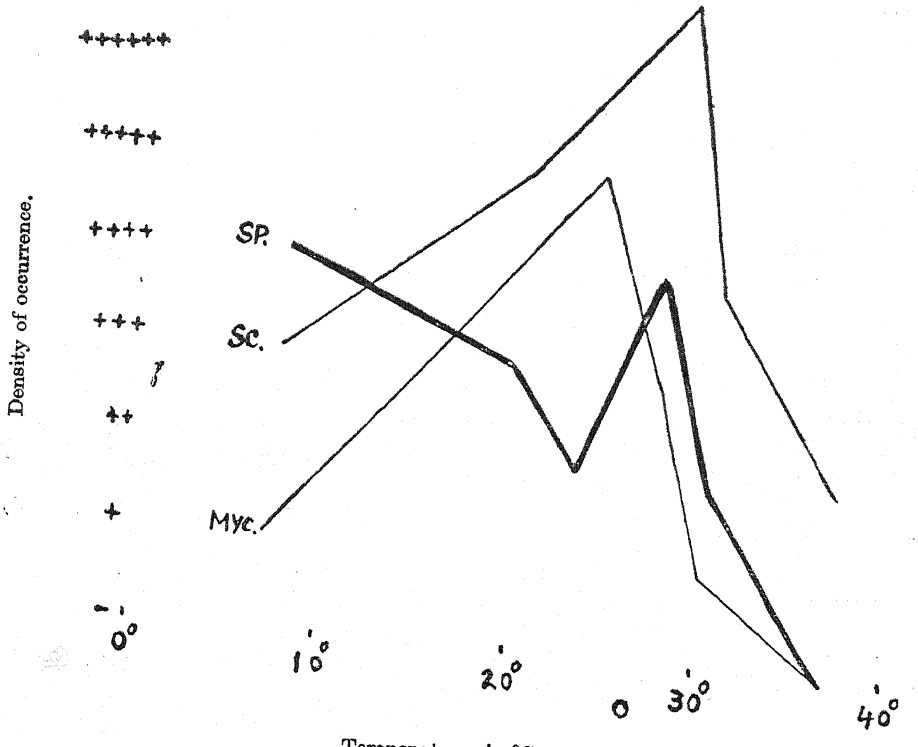

FIG. 1. Effect of various temperatures on growth and development of *Botrytis vulgaris*. Myc = spread of mycelium, Sc = sclerotial formation, and Sp = sporulation.

SUMMARY

1. A rot of grapes due to *Botrytis vulgaris* Fr. is recorded.

2. A brief etiology of the disease is given.

3. Spores of *Botrytis vulgaris* germinate readily in distilled water at 30°C. and produce one to three germ-tubes.

4. Formation of microconidia and anastomosis between two germ-tubes or hyphæ and germ-tubes was observed.

5. No ascigercus stage was found associated with this fungus in culture or in the fields.

6. Oat agar and Coon's agar were found to be very suitable media for the growth of *Botrytis vulgaris*.

7. Correlation of temperature and growth of *Botrytis vulgaris* was studied. 27°C. was found to be the optimum temperature for the growth of mycelium and sclerotial formation, while sporulation was most active at 23°C. At 37°C. the growth of aerial mycelium of the fungus and formation of spores were inhibited but some sclerotia were formed.

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SOME NOTES ON EYE-STRIPES IN ACRIDIDAE*

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ATTENTION has been drawn to the existence of stripes in the compound eyes of various species of Acrididae by systematists in their descriptions, though little significance seems to have been attached by them to this character. Roonwal [1936] was the first to notice in *Schistocerca gregaria* Forsk. (the desert locust), the existence of two types of individuals among the "solitaria" population found on the Mekran Coast—one possessing six stripes in the eyes, and the other, seven stripes. In his note, he observed: "The origin of this difference is, however, obscure. Whether this is inherited in a genetic way can only be shown by extensive breeding experiments." Subsequently, he studied the development of stripes in the hoppers of *Schistocerca* at Pasni, and came to the conclusion that, while in the six-striped type the number of eye-stripes increased at the rate of one for each moult, in the seven-striped one, two stripes were added in one of the hopper instars [Rao, 1938].

In the course of certain feeding experiments, however, Mukerji (working at Karachi) and Batra (stationed at Ambagh) had opportunities of following the development of a large number of hoppers, and their observations, made quite independently, showed that there was a perfect correlation between the number of moults passed through and the number of the stripes acquired by the hopper [Mukerji and Batra, 1938]. Thus in a five-instar life-cycle, an adult with six eye-stripes was produced, while a seven-striped eye was the result of the occurrence of an extra moult, or in other words, of a six-instar life-history. In a few cases, two extra moults were noticed, resulting in the production of adults with eight stripes in the eye. In a few other instances, Mukerji met with cases of a four-instar life-cycle accompanied by the production of a type with five eye-stripes.

Should these conclusions be confirmed, the eye-stripe character in *Schistocerca* would apparently serve as an easily recognisable index of the developmental history of the hopper stages of each individual, and may also serve

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to signify any possible tendencies in regard to change of phase. The senior writer had opportunities of examining a large series of specimens of *Schistocerca gregaria* gathered from actual swarms during past infestations in India, belonging to :—

(1) Karachi Locust Collections including *gregaria* forms collected in 1931 and a few specimens from Africa presented by Dr. B. P. Uvarov,

(2) Collections of the Imperial Entomologist, New Delhi (1904-1930),

(3) Collections of the Indian Museum, Calcutta (1889-1915), and

(4) Collections of the Professor of Entomology, Poona Agricultural College (1915-1930).

In all the *gregaria* forms subjected to scrutiny, he invariably found six stripes in the eyes (wherever the stripes were clearly visible), accompanied by 26 antennal segments in all such specimens as had the antennae intact. He also noticed that the specimens collected at Pasni and elsewhere during the time of the great incursion of locusts in July 1935, and the small incursion of May 1932 at Pasni [Rao, 1936] consisted predominantly of forms with six eye-stripes possessing *gregaria* ratios. In the course of biometrical work, the junior writer had ample opportunities of examining both *gregaria* and *solitaria* phase material of *Schistocerca* collected in the course of the last six years, from the various habitats of the desert locust within Indian limits. His observations go to show that collections of non-gregarious forms are generally made up of both six-striped and seven-striped types, though at certain times seven-striped ones were found predominating. The six-striped individuals, however, show mostly *solitaria* ratios, and usually possess 27 antennal segments, instead of the 26 found in *gregaria* forms. The seven-striped forms generally have 28 to 29 antennal segments, while eight-striped forms have 30 (as noted in a small number of specimens with eight eye-stripes found in nature in 1936). The dominance of the six-striped character in *gregaria* forms may, to a certain extent, be taken as an index of the rapidity of their developmental processes, resulting in the relative shortness of the hopper period as compared with that of the *solitaria* forms.

In certain other genera of Acrididae, in which eye-stripes are known to occur, a general correspondence of the number of stripes with the number of instars has been recorded in cases where the life-history has been worked out. In the case of *Patanga succincta* L. (the Bombay locust), eight eye-stripes have been noted to be quite common in the forms collected from swarms (1904-1910) ; and even among those met with in the desert in the course of locust surveys in recent years, eight-stripes are the general rule, though a few nine-striped forms have also been met with in the Rajputana desert. In addition, a few forms with seven eye-stripes have also been noticed in the collections. In this connection, it may be mentioned that Lefroy [1906] has recorded eleven cases of rearing of the Bombay locust from egg to adult,

of which nine hoppers passed through seven moults, while one underwent six and another eight moults. In the single specimen of *Nomadacris septemfasciata* Serv. (the Red-Locust of Africa), presented to the Karachi collection by the Imperial Institute of Entomology, London, seven eye-stripes are seen. which would correspond with the record of the general occurrence of six moults in its life-history [Faure, 1935]. In *Colemania sphenarioides* Bol. (the Deccan grasshopper), six to seven eye-stripes are found, which may be correlated with the occurrence of five or six instars recorded by Coleman [1911].

Six to seven vertical stripes are also to be found in the eyes of *Anacridium egyptium* L. (the Egyptian grasshopper), and in *Cyrtacanthacris tatarica* L. Similar prominent vertical eye-stripes are noticeable also in the following genera : *Caloptenopsis*, *Calliptamus*, *Thisoecetrus*, *Euprepocnemis*, *Dericorys*, *Spathosternum*, etc. Instead of straight and vertical stripes, curved, concentric ones are to be found in certain genera of the group *Pyrgomorphinae*, *viz.*, *Pyrgomorpha*, *Colemania*, and *Chrotogonus*. In *Acrida*, the stripes are parallel and horizontal (not vertical) in correspondence with the position of the eye in the anteriorly projecting snout of this form and may vary in number from seven to ten.

In certain Acrididae, stripes are conspicuous by their absence, the eye being generally irregularly marbled with cream and brown, as for example, in the genera, *Locusta* (*Locusta migratoria* L.), *Dociostaurus*, *Sphingonotus*, *Pyrgodera*, etc. In *Œdaleus*, however, stripes are visible in many specimens, though rather faintly.

In certain other genera, only two or three anterior stripes are clearly visible, the posterior ones becoming more or less masked by light brown, as, for instance, is the case with *Hieroglyphus*, *Catantops*, *Oxya*, and *Choroedocus*.

In *Hieroglyphus banian* F., an examination of a great number of live hoppers of all stages in the field in regard to the eye-stripes as well as the number of their antennal segments gave ample indications that, as in *Schistocerca*, the number of stripes progressively increases in this form with the number of moults passed through, it being noticed that the eye-stripes were generally clearer and more distinct in the hopper stages of *Hieroglyphus* than in the adult forms.

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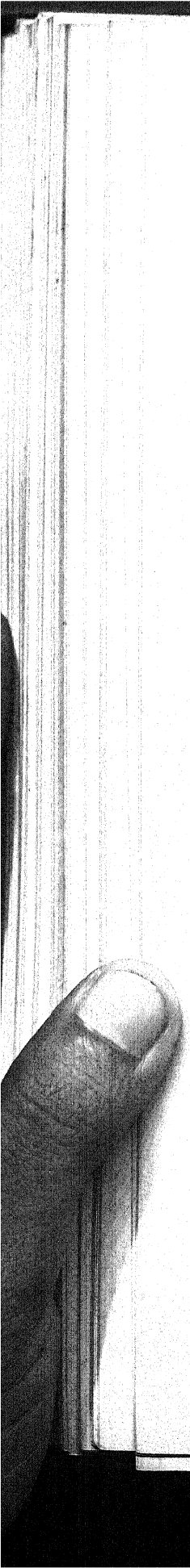

DELINTING AND GERMINATION OF COTTON SEEDS AS AFFECTED BY $ZnCl_2.2HCl$ TREATMENT

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(With four text-figures)

PRELIMINARY to cotton cultivation, two problems confront its grower, *viz.*, (*i*) how to delint the seeds, and (*ii*) how to secure earlier and more regular germination. The dung treatment of fuzzy types, a common practice prevalent among the Indian peasants, though it helps a clear broadcast, does neither delint the seed nor affect the germination process. The presence of fuzz on seeds handicaps germination and also interferes in the isolation of dead and shrivelled seeds from the stock.

The importance of good germination is unique and in fact this phase is emphatically the most critical one in the plant's life-span. It is here that the seeds either perish because of limited moisture or rot due to excessive irrigation. A prompt and complete germination is, therefore, an essential preliminary to the future prosperity of plants, for it ensures, amongst other things, a regular full stand, a complete elimination or reduction of weeds, a protection against heat of the sun and a uniformity in their growth. To gain this end, treatments like scarification [Rose, 1915], alteration in temperature [Harrington, 1923; Morinaga, 1926], irradiation [Singh and Choudhri, 1938] and immersion in chemicals [Denny, 1926; Archibald, 1927] have been attempted by various investigators.

Unlike other crops, in cotton, a method that would supply the two essential needs detailed above would be of genuine economical worth and any step towards the achievement of this end would be an important addition to the success in the cultivation of cotton. Assuming that 90 per cent of cotton fibre consists of pure cellulose [Miller, 1931] which readily dissolves in $ZnCl_2.2HCl$ [Cohen, 1925; Miller, 1931], the present investigation was designed to test the validity of this method of seed treatment towards delinting and obtaining a more successful germination.

Method

Pure strains of two cotton varieties, *viz.*, C402 (Indian) and Perso-American, after careful grading for uniformity, were soaked in an acidic solution of zinc chloride (zinc chloride dissolved in twice its weight of concentrated hydrochloric acid) contained in a pneumatic trough for durations of 2, 5, 10, 15, 20, 30 and 60 minutes respectively. To ensure homogeneous action of the solution on seeds they were constantly stirred with the aid of a thick glass rod. At specified intervals lots of seeds were removed from the container with a glass spoon, and the acid solution drained off. The various lots were then transferred to sieves and subsequently washed under jets of running water for periods of not less than half an hour to remove completely the adhering acid. To provide basis for comparison of results, two types of checks were maintained (*i*) dry and (*ii*) soaked. The set of dry seeds was not subjected to a y pre-sowing treatment while the seeds belonging to the other were soaked in water for half an hour to balance the effect of water washing in case of treated seeds. Since $ZnCl_2.2HCl$ solution contained no water, a water-soaked control for duration of more than half an hour was considered without use. The external moisture from both the treated and the soaked control was wiped off by putting the seeds in the folds of blotting papers. After another ten minutes the seeds were sown in big enamelled dishes under sand culture. The use of sand was preferred to ordinary soil in order to eliminate the effect of heterogeneity of the growing substratum, which would otherwise affect the experimental observations.

In all, ten sets of experiments were conducted with each of the two cotton varieties. In each set ten dishes were employed and in each dish 25 seeds were sown. The various lots were examined every morning and evening and germination counts made which for a tacit appreciation of the data were computed per hundred and analysed statistically using each dish as a replication.

Results

Delinting.—Cotton fibre furnishes the best example of almost pure normal cellulose, hygroscopic in character and insoluble in ordinary solvents. Concentrated sulphuric acid is said to dissolve it but gradually [Morinaga, 1926]. The dissolution of fuzz was secured more readily on treatment of seeds with $ZnCl_2.2HCl$ solution. It depended, however, to a great extent upon the variety of cotton investigated. In type C402, for instance, about three-fourths of the fuzz dissolved only after two minutes' soaking, while complete delinting occurred after five minutes' duration. Perso-American belonging to *hirsutum*, which is noted for its larger surface and greater fuzz than the *desi*, required obviously a longer treatment of ten to fifteen minutes' duration for the entire removal of fuzz from seeds. In this case treatment up to five minutes was found to be insufficient to bring about this change. Approximately one-fourth of the fuzz after two minutes' immersion of seeds and about half or

slightly more after five minutes dissolved in $ZnCl_2.2HCl$ solution, which indicated the differential response of the two varieties, possibly due to differences in the strength of fuzz and floss.

Germination effects.—In order to know the comparative details of seed germination of the two types of cotton, the counts made every morning and evening on both the controls and the treated samples are presented in detail in Table I.

TABLE I

Comparative changes in germination of control and treated cotton seeds belonging to types C402 and Perso-American

Treatment	No. of seeds	Number germinated after an interval of (days)								Total	Germination capacity (percentage)	Duration of germination (days)
		½	1	1½	2	2½	3	3½	4			
					C 402							
Control (dry) . .	250	0	100	53	20	10	10	0	0	193	77·2	3
Control (soaked) . .	,,	0	27	28	37	5	3	0	0	100	40·0	3
$ZnCl_2$·2HCl 2 mins. .	,,	80	125	35	3	0	0	0	0	243	97·2	2
,, 5 mins. .	,,	72	128	45	0	0	0	0	0	245	98·0	1½
,, 10 mins. .	,,	78	133	32	0	0	0	0	0	243	97·2	1½
,, 15 mins. .	,,	17	188	15	10	0	0	0	0	230	92·0	2
,, 20 mins. .	,,	0	193	23	0	2	0	0	0	218	87·2	2½
,, 30 mins. .	,,	0	132	33	17	13	0	0	0	195	78·0	2½
,, 60 mins. .	,,	0	125	33	20	10	0	0	0	188	75·2	2½
					Perso-American							
Control (dry) . .	250	0	0	18	5	15	15	7	0	60	24·0	3½
Control (soaked) . .	,,	0	38	58	33	7	12	5	0	153	61·2	3½
$ZnCl_2$·2HCl 2 mins. .	,,	12	38	53	35	5	10	2	0	155	62·0	3½
,, 5 mins. .	,,	12	43	65	38	5	7	0	0	170	68·0	3
,, 10 mins. .	,,	15	67	43	45	8	17	0	0	195	78·0	3
,, 15 mins. .	,,	0	52	53	15	30	23	0	0	173	69·2	3
,, 20 mins. .	,,	3	42	58	12	17	13	5	0	150	60·0	3½
,, 30 mins. .	,,	0	43	42	20	22	5	3	0	135	54·0	3½
,, 60 mins. .	,,	0	35	48	12	10	13	0	0	118	47·2	3

It is well indicated that external emergence of the embryo did not take place simultaneously in all the sets and naturally, therefore, the percentage germination was to be affected. Even in those cases where sprouting began at the same time and on the same day, this aspect greatly differed from set to set. It was experienced that lots, where germination startedearlier or where

a good many seeds germinated at the commencement of the process, usually gave good account of themselves at the end. With a view to determining then the degree of reliance on such variations arising out of treatments, the essential data are subjected to critical statistical test and analysis of variance worked out as shown in Table II.

TABLE II

Analysis of variance of germination percentage

Due to	Degrees of freedom	Sum of squares	Mean square	Ratio of variances
C402				
Blocks . .	9	637·1	70·78	..
Treatments . .	8	26969·6	3371·20	83·27
Error . . .	72	2914·9	40·48	..
Total .	89	30521·6	..	..
Perso-American				
Blocks . .	9	730·0	81·11	..
Treatments . .	8	19440·3	2430·00	78·38
Error . . .	72	2235·1	31·02	..
Total .	89	22405·4	..	..

According to the above scheme, the ratio of variance due to treatments in both types of cotton is significant even at 1 per cent level. This would indicate that the differences noticed in seed germination were not mere chance variations but were the actual after-effects of seed treatment with $ZnCl_2.2HCl$.

Out of the many cases thus experimented, those exhibiting the most pronounced variations from day to day in the two types of cotton have been plotted as daily total percentages (Figs. 1 and 2) to indicate more clearly their departure from the normal.

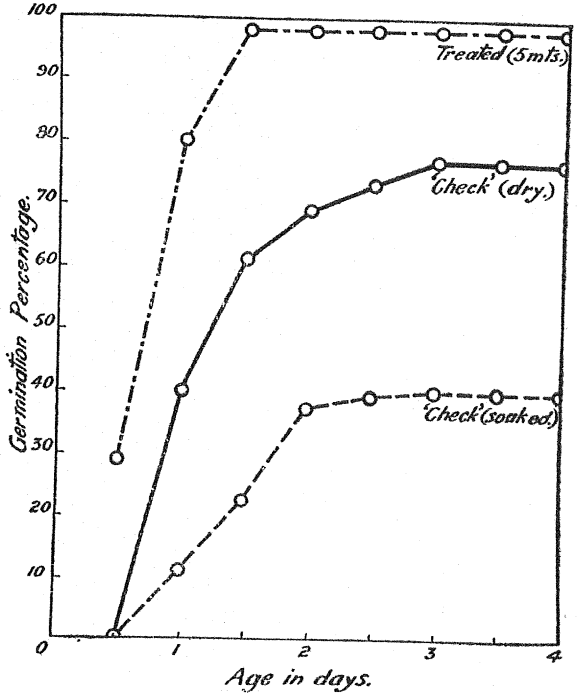

FIG. 1. Total germination percentages of control and treated cotton seeds (*Var.* C402) on days following sowing

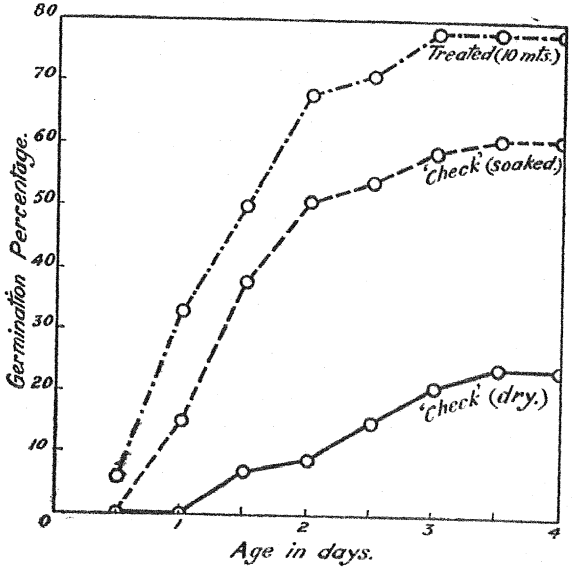

FIG. 2. Total germination percentages of control and treated cotton seeds (*Var.* Perso-American) on days following sowing

Whereas it is evident that the treated lot began to germinate earlier and had a higher initial start, it is also noticed that the total percentage figures in the treated set by subsequent additions maintained consistently a level higher than the control. This is true for the majority of the doses tried in this investigation though the degree of change was far too less in some cases than in others (Table I). Some heavier doses, for instance, those of one hour duration, did not yield promising data in this regard.

A careful examination of Figs. 1 and 2 further showed that soaking of seeds in water above had a profound influence upon their capacity to germinate, but this effect on the two types of cotton was dissimilar. The American variety showed better germination than its dry control, while C 402 was adversely affected. The treated samples deviated so significantly from both types of checks that the ultimate effects could not be attributed to water treatment.

For evaluating the relative significance of germination data obtained with regard to different treatments, critical difference has been computed in each of the two cases and percentage counts arranged in descending order as shown in Table III.

TABLE III

Treatments arranged in order of effects produced

C402

Critical difference = 7·49

Treatments (mins.)	5	2	10	15	20	30	Control (dry)	60	Control (soaked)
Germination percentage	98·0	97·2	97·2	92·0	87·2	78·0	77·2	75·2	40·0

Perso-American

Critical difference = 6·34

Treatments (mins.)	10	15	5	2	Control (soaked)	20	30	60	Control (dry)
Germination percentage	78·0	69·2	68·0	62·0	61·2	60·0	54·0	47·2	24·0

From the above table it is clear that most of the treatments produce significant effects with respect to dry control, while those ranging from two to fifteen minutes' duration are specially noted for their high level of significance.

Another fact of importance that could not escape notice was that the curves exhibiting total germination percentage for the treated lots (Figs. 1 and 2) were characterised by greater steepness in the earlier than the later stages.

This fact was more clearly exhibited by the curves for Indian cotton than those for the American, where the germination was comparatively gradual. A perusal of Table I would further strengthen the point. In the case of C402 over 50 per cent of germination occurred in every one of the treated sets within the first 24 hours of sowing inasmuch as some lots exhibited even 80-84 per cent. Thus, lots showing very high germination on the first day completed the same within another twelve hours' time after which germination was constant in these sets. On this account such curves were almost abrupt against the more smooth ones of the control where the percentage germination was gradual though less, and covered a much longer duration.

Considering the data collectively in several useful directions, *e.g.*, speed, duration and capacity of germination (Table I), it is noted that the suitable doses with regard to type C402 were those of two to ten minutes' duration, that of five minutes yielding the best results. Such doses hastened germination, took a much shorter time to complete it, and initiated a very high percentage of seed to sprout as compared with "checks". The same was true for American cotton as well, where treatments for five to fifteen minutes were more satisfactory than others while the one applied for ten minutes induced the best results. Below and beyond the best dosages there was a gradual diminution in the percentage capacity of germination of seeds which sprouted comparatively late and took a longer period to finish it. The great efficacy of the treatment of cotton seeds with $ZnCl_2.2HCl$ was, however, evidenced by the readiness with which this solution acted even after two minutes' application.

In American cotton, the percentage of germination was usually greatly enhanced, even as much as 54 per cent as compared to its dry control while the duration was not much affected. In Indian cotton, on the contrary, though the percentage increase was only 20-21, the speed of development was high and the duration of germination almost halved. In fact the latter type is more acclimatised to the conditions of this country and hence as many as 77 per cent of the seeds germinated even without any treatment, with much less scope left for such treatments. Smaller increase in the percentage germination of type C402 following $ZnCl_2.2HCl$ immersion, therefore, should not be attributed to the inefficiency of this solution. Rather, from many other view-points (*cf.* heights and dry matter accumulations) it appeared that type C402 was more advantageously affected by this pre-sowing treatment than Perso-American.

Addendum

While the object of the present work was solely to determine the effectiveness of this method of seed treatment towards germination, which phase in itself is the most important and critical one, measurements of the seedling growth were also made to know whether the effects produced, if any, were transitory or permanent. A large number of plants from each of the lots (which rendered fortuitous fit of observation very unlikely) were examined with regard to their heights, accumulation of dry matter and other essential details. The average data obtained from these measurements have been represented graphically in Figs. 3 and 4.

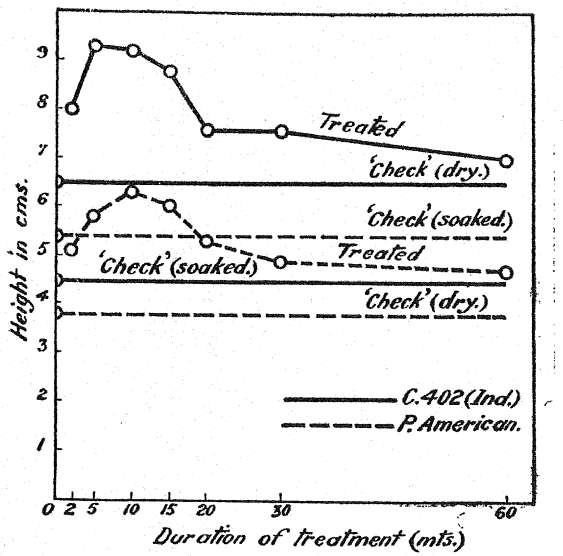

FIG. 3. Average heights of one week old cotton seedlings, shown to express the results on a comparative basis with regard to the duration of treatment provided. The 'checks' are indicated by straight lines

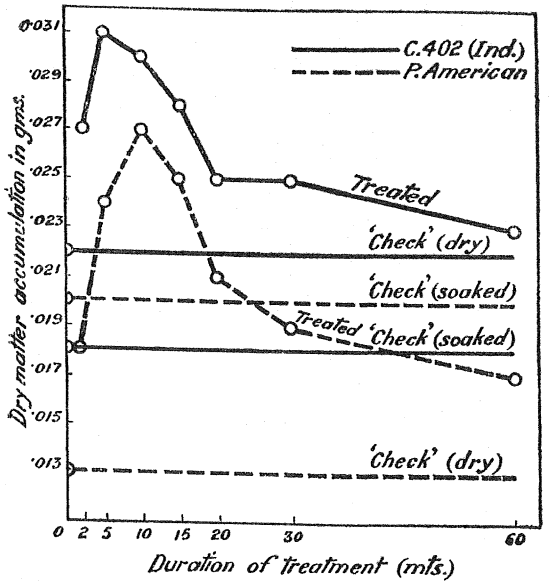

FIG. 4. Dry matter accumulation of one week old average cotton seedlings, shown to express the results on a comparative basis with regard to the treatment provided. The 'checks' are indicated by straight lines

It was observed that a number of control seedlings showed poor development, drooping habit and scant growth. Also, a fair percentage of mortality amongst them was evident after some time with little or no protrusion of the plumule. In treated sets, particularly those the seeds of which were subjected to five, ten or fifteen minutes' immersion, plants were invariably healthy, erect, firm and had a better stand. Such plants were also taller (Fig. 3) and accumulated greater dry matter (Fig. 4) than their respective controls. Of the types of cotton thus investigated, treated seedlings of C402 were radically different from their controls. On the whole, the general trend of these observations was very much like those noted on germination.

Summary

The data recorded in this paper were gathered to estimate the degree of delinting and germination effects in Indian and American varieties of cotton following seed immersion for various durations in $ZnCl_2.2HCl$. The experiments were conducted in sand culture and the enquiry which was proposed in the first instance to be restricted to the germination phase alone was later extended to the determination of seedling growth as well.

The solution used in this work readily dissolved the fuzz present on cotton seeds. The seeds of American type were found to be slightly more resistive where for complete isolation of the fuzz, 10 to 15 minutes' soaking was essential against five minutes in the case of type C402. Doses shorter than five minutes were insufficient to cause complete delinting.

The germination process was greatly activated by $ZnCl_2.2HCl$ treatment of seeds. Doses of five to fifteen minutes' duration were very satisfactory in this regard. Such treatments initiated early start, higher percentage and early completion of germination. Shorter or longer treatments induced gradual depletion in the germinating vigour of seeds from the optimal value, though in the majority of cases germination was still superior to the controls.

The permanency of the effects of these treatments was well evidenced by the resulting seedlings which were taller and accumulated greater dry matter than their controls.

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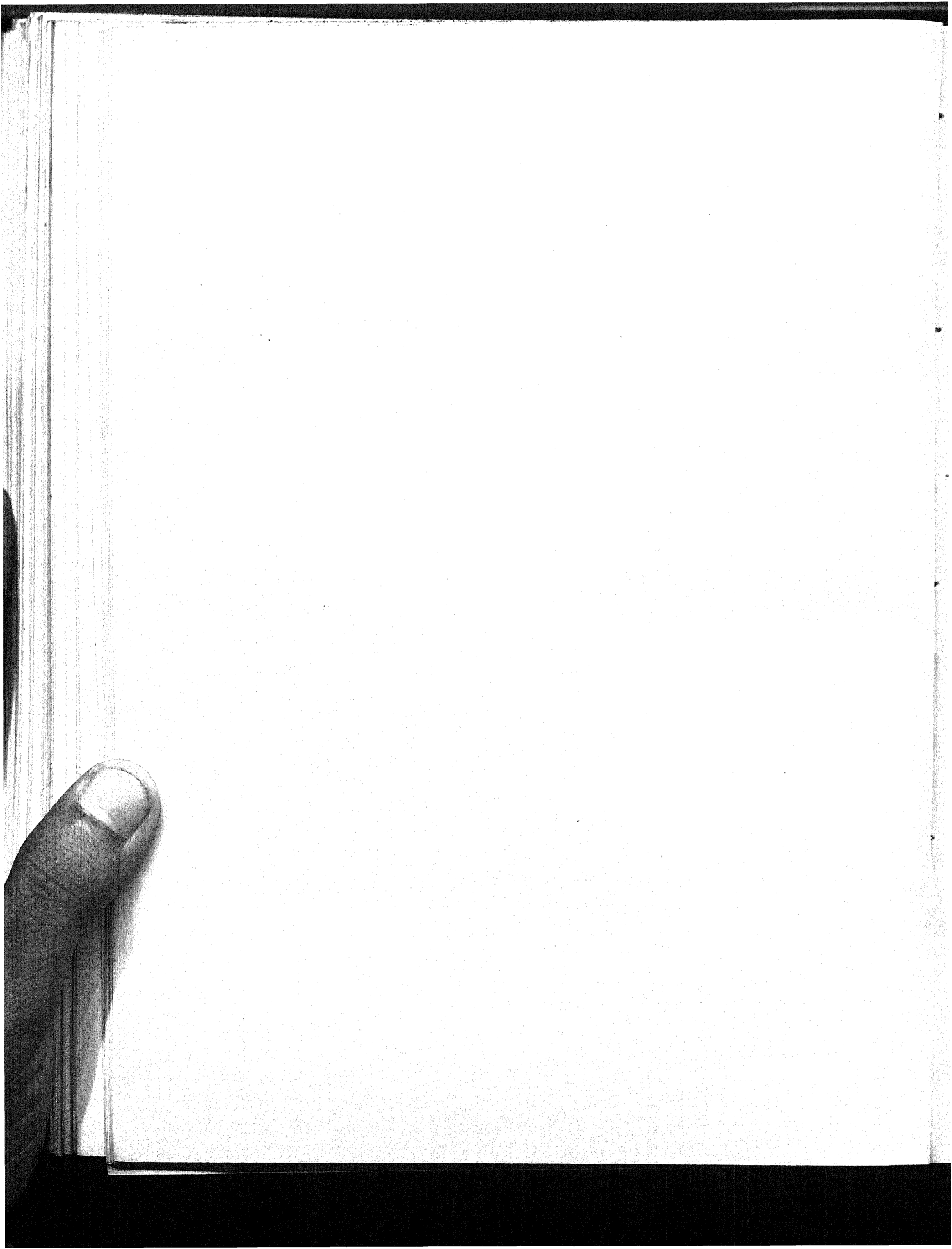

THE HOT FERMENTATION PROCESS FOR COMPOSTING TOWN REFUSE AND OTHER WASTE MATERIAL*

I. INTRODUCTORY

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SINCE Hutchinson and Richards [1921] first worked out the scientific principles underlying the conversion of straw and other organic matter into synthetic farmyard manure, the question of composting waste materials—agricultural or town wastes—has been attracting a good deal of attention both in this country and abroad. Based on the essential principles of Hutchinson and Richards, but involving differences in technique to suit local climatic and labour conditions, the nature of the raw material, etc., various processes have been standardised for the preparation of compost—*e.g.*, the "Adco" process of Richards [1921], the Indore process of Howard and Wad [1931], the "activated compost" process of Fowler [1934], etc. Common features of the above methods are :

(a) that they aim at a rapid and efficient disintegration of the original material by the addition of a suitable nitrogenous starter so as to promote rapid microbial development ;

(*b*) that the decomposition is essentially aerobic and, generally, the compost is turned over a number of times to promote aeration ; in some cases, even underground flues are provided to increase air supply to the inner portions of the heap [Eden, 1938] ; and

(*c*) that the final aim is to obtain a well decomposed material, friable and easily broken down to a finely divided condition, generally brown to black in colour and with a C : N ratio near 10 :1.

Few systematic studies have been reported of the carbon and nitrogen transformations taking place in heaps composted under large-scale conditions of farm or town practice, and of the ultimate gains or losses of nitrogen occurring during such composting process. The accuracy of the data has, in general, been vitiated by the heterogeneous nature of the waste material—be it farm or

*The investigations reported in this and the following papers of this series were carried out at the Indian Institute of Science, Bangalore, under a scheme 'for the preparation of cheap synthetic manure from town refuse and other waste material' financed by the Imperial Council of Agricultural Research.

town waste—and the difficulty of sampling involved while dealing with cart-loads of such heterogeneous material. The data, however, that have so far been presented [Ayyar, 1928, 1933] ; [Jackson and Wad, 1934] ; [Jackson, Wad and Panse, 1934] ; [Rao and Subrahmanyan, 1935] ; [Rao, 1936] have shown that in the ordinary methods of aerobic composting adopted in this country and elsewhere, heavy losses of carbon and of nitrogen occur to the extent of 40 to 60 per cent of the amounts originally taken.

The loss of nitrogen is particularly serious in the composting of town wastes, *e.g.*, street sweepings and night soil, the latter of which possesses a C : N ratio near 8 : 1. It is difficult to widen the C : N ratio of such a compost heap to the desired level of about 30 : 1 at the start, since the street sweepings in India contain a good proportion of leaves which are also rich in nitrogen. Under these conditions, the loss may easily amount to over 50 per cent of the nitrogen originally taken. Rao and Subrahmanyan [1935] found that when town-wastes were sprayed with emulsified or previously liquefied night soil over a number of days, the nitrogen content rose up to 1·2 per cent in the case of composts treated with night soil emulsion and to 0·7 per cent. when liquefied night soil was used. On making further additions of nitrogen on subsequent days, the nitrogen content remained stationary at the above levels, indicating a complete loss of all the added nitrogen. Losses of nitrogen from 20 to 40 per cent are reported by Ayyar [1928, 1933] and Rao [1936] even while using inorganic nitrogenous starters such as calcium cyanamide, ammonium sulphate or sodium nitrate for composting purposes, in place of night soil.

Apparently, the aerobic conditions of decomposition adopted in the above cases favoured excessive losses of nitrogen and of organic matter, especially when the mass was turned over at intervals to promote humification. A modification, wherein the access of air could be controlled to a greater extent, but at the same time the humification of the material may be allowed to proceed, offered hopes of a better conservation of nitrogen and of organic matter.

A method which promised to yield satisfactory results in respect of the above requirements was the hot fermentation method, first brought to public notice by Krantz [1925], who successfully incorporated it in his well-known Edelmist process for the preparation of farmyard manure.

Krantz recommended special equipment and a definite arrangement of packing the material and devised his method originally for the efficient storage and ripening of farmyard manure. Various workers [Ruschmann, 1927 ; Boctskaul, 1927 ; Fruwith 1928 ; *vide* also Cunningham, 1927 ; Jenkins, 1935] have since tried the method for the production of farmyard manure from dung and stall litter and have claimed a higher conservation of nitrogen and of dry matter and a higher crop response for the method than is obtainable by the aerobic methods usually adopted.

The essential principle underlying the hot fermentation process is that the material is allowed to decompose aerobically, under optimum conditions of moisture and nitrogen supply, for a limited period (about five or six days)

during which time rapid decomposition takes place and a high temperature (60° to 70°C) is reached. When the temperature begins to fall, the supply of air is cut off by compacting the mass and pressing it down with a layer of fresh material or by soil or otherwise.

Preliminary trials carried out by Shrikhande *et al.* [1936] at Bangalore showed that the hot fermentation method was peculiarly suited to the composting of town wastes with inorganic nitrogenous starters such as ammonium sulphate, calcium cyanamide, sodium nitrate or organic starters such as night soil. The data obtained (*vide* Table I) showed that there was complete recovery of nitrogen by the method, except in the case of calcium cyanamide, when the initial C : N ratio was 30 : 1 ; but there was increasing loss at narrower ratios, especially at 10 : 1. The losses in dry matter, in the hot fermentation method were of the order of 15 to 20 per cent. The high temperatures attained during the process served to destroy fly-larvae and pathogenic organisms, and the final product obtained, even when night soil was the base, possessed no bad odour.

TABLE I

Composting of town refuse

Form of nitrogen added to refuse	C : N ratio at the commencement											
	30 : 1				20 : 1				10 : 1			
	Dry matter recorded	Total nitrogen before composting	Total nitrogen after composting	Per cent nitrogen in the finished product	Dry matter recorded	Total nitrogen before composting	Total nitrogen after composting	Percent nitrogen in the finished product	Dry matter recorded	Total nitrogen before composting	Total nitrogen after composting	Per cent nitrogen in the finished product
	lb.	lb. oz.	lb. oz.		lb.	lb. oz.	lb. oz.		lb.	lb. oz.	lb. oz.	
Ammonium sulphate . .	216	3 12	3 13	1·79	209	5 12	5 3	2·49	205	8 12	7 8	3·62
Sodium nitrate . . .	216	3 12	3 13	1·82	237	5 12	4 14	2·13	240	8 12	6 3	2·74
Calcium cyanamide . .	207	3 12	2 9	1·19	228	5 12	5 0	2·06	224	8 12	6 0	2·45
Night soil	224	4 9	4 8	1·47	...	...	...	...	...	...	...	...

Each cistern contained originally 250 lb. of refuse

In view of the encouraging results obtained in the preliminary trials, it was deemed advisable to examine the method more critically ; to study the various factors influencing the efficacy of the process and to standardise the conditions for securing optimum decomposition ; to compare the hot fermented

compost with that obtained by aerobic methods in regard to manurial efficiency, by pot and plot trials as well as by laboratory analysis and nitrification tests. The results of such investigations are presented in the succeeding papers of this series.

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ESTIMATION OF POTASH IN SOILS BY THE COBALTINITRITE METHOD

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In this paper an attempt has been made to test the efficiency of the cobaltinitrite method—volumetric and gravimetric—for the estimation of potash in pure salts and in hydrochloric and citric acid extracts of soils. In computing the results, the formula, $K_2NaCo(NO_2)_6.H_2O$ of Addie and Wood [1900] has been employed. The results have been frequently checked with those obtained by the standard platinichloride (Lindo-Gladding) and perchlorate methods.

The method of Addie and Wood [1900] has been tested and modified previously by several workers, chiefly by Drushel [1907, 1908], Bowser [1909], Dennet [1929], Milne [1929], Van Rysselberg [1931], Hibbard and Stout [1933], Lewis and Marmoy [1933] besides others; but due to variations in the technique adopted by most of them, especially with regard to the method of precipitation, the preparation of the reagent, the concentration of the solution at the time of adding the reagent, the length of time allowed before the filtration of the precipitate, the nature and reaction of the potassium permanganate and the final treatment of the precipitate which differed in composition under certain conditions, the validity of the theoretical factor for potash based on Addie and Wood's formula was doubted. An elaborate study of the several methods indicated that with more constant conditions of precipitation and titration of the resulting compound with neutral, instead of acid—potassium permanganate—as in the case of Drushel's procedure, concordant results could be obtained by adopting Addie and Wood's formula. It was also found that the cobaltinitrite method gave as consistent results as the other accepted ones and was at the same time considerably quicker. Removal of basic and acid radicals such as calcium, magnesium, sulphates, phosphates, etc. was found to be unnecessary.

The preparation of the reagent and the details of the various experiments conducted, with the results obtained under each, are set forth below.

EXPERIMENTAL

Preparation and properties of the cobaltinitrite reagent

The reagent was prepared according to the method of De-Koninck [1899] as detailed below :— $Co(NO_3)_2$

Thirty grams of cobalt nitrate are dissolved in 60 c. c. of water and mixed with 100 c. c. of saturated sodium nitrite solution (about 50 grms. in 100 c. c.

water). The mixture is then treated with 10 c. c. of glacial acetic acid and warmed for a while over a steam oven or water bath. The nitrous fumes evolved are then evacuated by means of a filter pump and the whole allowed to settle for 24 hours. The reagent is then filtered and kept in the dark in a coloured bottle. It is more sensitive than platinum chloride: a drop of 0·1 per cent potassium chloride solution in 5 c. c. of water will give the reaction for potassium immediately the reagent is added. The reagent is quite stable for a fortnight but after this period it is not reliable.

Experiment 1

Estimation of potash in pure salts.—As a preliminary to the final application of the method to soils and artificial mixtures of potash, it was tested with pure analytical reagents with special reference to the influence of common acid radicals on potash estimations. The technique adopted for the volumetric estimation was essentially that of Drushel [1907, 1908] which in brief is as below :—

The solution containing potash is treated with an excess of cobaltinitrite reagent prepared according to De-Koninck [1899] and the whole mixture evaporated to a pasty consistency in acetic acid medium on a water bath. The precipitate, after washing with 10 per cent acetic acid, is oxidised directly with standard $N/10$ or $N/20$ potassium permanganate and the potash estimated by adopting the factor 1 c. c. $N/10$ potassium permanganate = 0·0008564 grm. of potassium oxide.

For the gravimetric estimation, the precipitate is dried to constant weight in an air oven at 120°C. and the potash estimated by applying the conversion factor 0·2074 based on Addie and Wood's formula namely, $K_2NaCo(NO_2)_6 .H_2O$.

The results obtained for the various salts including potassium sulphate by the cobaltinitrite, gravimetric and volumetric procedures, as well as by the official platinichloride method (Lindo-Gladding) are given in Tables I and II, along with the percentage recovery of potash (K_2O) under each, taking the theoretical value as the standard.

TABLE I

Recovery of potash from pure salts by the platinichloride and Cobaltinitrite (volumetric and gravimetric) methods

Salts used for analysis	K_2O theoretical value mg.	K_2O by the cobaltinitrite method. Average of four determinations		K_2O by the platinichloride method. Average of four determinations mg.	K_2O recovered by the Cobaltinitrite method		K_2O recovered by the platinichloride method per cent
		Gravimetric mgms.	Volumetric mgms.		Gravimetric per cent	Volumetric per cent	
1. Potassium chloride A. R. (KCl)	53·2	52·8	53·0	52·9	99·25	99·62	99·43
2. Potassium phosphate A. R. (KH_2PO_4)	31·2	31·1	31·1	31·0	99·68	99·68	99·36
3. Potassium nitrate A. R. (KNO_3)	38·0	37·7	37·8	37·8	99·21	99·47	99·47
4. Potassium sulphate A. R. (K_2SO)	44·1	43·9	44·0	43·8	99·55	99·75	99·32

TABLE II

Influence of common acid radicals on the volumetric determination of K_2O by the Cobaltinitrite method

Salts used for analysis	K_2O theoretical value mg.	K_2O estimated, average of four determinations mg.	Deviation of the estimated value from the theoretical mg. + or —	K_2O recovered per cent
1. Potassium chloride A. R. (KCl) .	53·20	52·90	—0·30	99·44
" " " " .	5·32	5·30	—0·02	99·62
2. Potassium phosphate A. R. (KH_2PO_4)	31·20	31·00	—0·20	99·36
" " " "	3·12	3·14	0·02	100·61
3. Potassium nitrate A. R. (KNO_3) .	38·00	37·80	—0·20	99·47
" " " " .	3·80	3·77	—0·03	99·21
4. Potassium sulphate A. R. (K_2SO_4) .	44·10	43·90	—0·20	99·55
" " " " .	4·41	4·38	—0·03	99·32

The figures show that the recovery of potash by the cobaltinitrite methods —volumetric and gravimetric—is not only quantitative but also agrees very closely with that of the standard platinum chloride procedure. To test the accuracy of the formula of Addie and Wood adopted during the course of this investigation the following indirect experiment was conducted.

Experiment 2

Ten c. c. aliquots of pure potassium sulphate (K_2SO_4 A. R.) solution equivalent to 0·043 grm. potassium oxide were treated with 10 c. c. of cobaltinitrite reagent and the ratio of K : Na in the medium varied from 1 : 30 to 1 : 200 by adding pure sodium chloride solution. The precipitates obtained were dried at 120°C. to constant weights and the potassium oxide calculated from Addie and Wood's formula. The dried precipitates were then dissolved in hot dilute hydrochloric acid and the potash estimated in the resulting solutions by the perchlorate method. The results obtained by both the methods are recorded in Table III. It may be seen that the results agree very closely with one another, thereby confirming the correctness of the formula, in spite of the wide variations in the ratio of K : Na from 1 : 30 to 1 : 200.

TABLE III

Gravimetric estimation of K_2O by the cobaltinitrite and perchlorate methods

Amount of K_2O in the medium (theoretical value) (Mg.)	Ratio of K : Na	K_2O recovered by		Per cent error in K_2O recovery by	
		Cobaltinitrite method (average of four estimations) (Mg.)	Perchlorate method (average of four estimations) (Mg.)	Cobaltinitrite method + or—	Perchlorate method + or —
K_2SO_4—10 c. c. equivalent to 43·2 mg. K_2O . .	1 : 30	43·3	43·1	+0·23	—0·23
Ditto . .	1 : 40	43·0	43·4	—0·46	+0·46
Ditto . .	1 : 50	43·1	43·3	—0·23	+0·23
Ditto . .	1 : 60	42·9	43·0	—0·70	—0·46
Ditto . .	1 : 75	43·3	43·2	+0·23	*Nil*
Ditto . .	1 : 100	43·0	43·3	—0·46	+0·23
Ditto . .	1 : 200	42·9	43·1	—0·70	—0·23

Experiment 3

This was carried out to study the conditions affecting precipitation of potash, and the subsequent treatment of the precipitate. The results obtained under different conditions are recorded in Table IV.

TABLE IV

Potash estimation by the cobaltinitrite (volumetric) method under different conditions of precipitation and treatment of the precipitate

Nature of precipitation and treatment of the precipitate	K_2O (theoretical) (Mg.)	K_2O recovered (average of four determinations) (Mg.)	Deviation of the estimated value from the theoretical (Mg.)	Percentage error of K_2O recovered + or —
1. Solution containing potash evaporated to a pasty consistency, the precipitate treated with $KMnO_4$ acidified with 1 : 1 H_2SO_4 (10 c. c. for every 200—300 c. c. of $KMnO_4$ as finally diluted) . . .	21·6	23·2	1·6	+7·40
2. Evaporated the potash solution to a pasty consistency, treated the precipitate with cold $KMnO_4$, raised the whole to a boil and then added 10 c. c. of 1 : 1 H_2SO_4 as in 1. .	21·6	21·8	0·2	+0·92
3. Solution containing potash concentrated to 15—20 c. c.; Cobaltinitrite reagent added to the cold solution and the mixture allowed to stand for 24 hours; the precipitate then treated with neutral $KMnO_4$ at the boiling point, followed by the addition of 10 c. c. of 1 : 1 H_2SO_4 as in 1 and 2	21·6	21·9	0·3	+1·39
4. Potash solution evaporated to a pasty consistency; precipitate added to neutral $KMnO_4$ at the boiling point and subsequently to H_2SO_4 (1 : 1) as in 1 and 2 . .	21·6	21·4	0·2	—0·92

It is evident from the table that the treatment of the precipitate with potassium permanganate acidified previously affects the results considerably, the percentage error being as high as 7, if the usual factor, 0·0008564 gram potassium oxide is used for every c. c. of a deci-normal potassium permanganate. This difference in value may be due to the oxidation of the precipitate with acid permanganate instead of neutral, thereby affecting the potassium equivalent of K_2O. In such cases, a new empirical factor has to be worked out to get at the real value of potassium oxide as has been done by Milne [1929].

Experiment 4

Influence of common bases on potash estimation.—The individual influence of iron, alumina and magnesium added as sulphates and calcium in the form of carbonate on potash values was studied by keeping the potash constant and varying the quantities of bases. The results presented in Table V go to prove the reliability of the method even under different conditions. The mixtures were prepared to conform to the minimum and maximum quantities of bases that might be present in 50 c. c. of hydrochloric acid extract of 20 grms. of ignited soil made up to 500 c. c. The percentages of iron and alumina have been varied from 10 to 50 each and lime and that of magnesia from 0·1 to 10.

TABLE V

Influence of bases on the volumetric estimation of K_2O by the cobaltinitrite method

Metalic salts used	K_2O added (theoretical) (Mg.)	K_2O recovered (average of four determinations) (Mg.)	Deviation of the K_2O recovered from the theoretical value (Mg.) + or —	Per cent. error of K_2O recovered + or —
I				
Ferrous sulphate ($FeSO_4.7H_2O$) equivalent to—				
1. 0·25 gm. Fe_2O_3	21·60	21·76	+0·16	+0·74
2. 0·5 gm. Fe_2O_3	21·60	21·78	+0·18	+0·83
3. 0·75 gm. Fe_2O_3	21·60	21·44	—0·16	—0·74
4. 1·0 gm. Fe_2O_3	21·60	21·30	—0·30	—1·39
II				
Aluminium sulphate [$Al_2(SO_4)_3.18H_2O$] equivalent to—				
1. 0·25 gm. Al_2O_3	10·8	10·90	+0·10	+0·93
2. 0·5 gm. Al_2O_3	10·8	11·00	+0·20	+1·85
3. 0·75 gm. Al_2O_3	10·8	10·80	*Nil*	*Nil*
4. 1·0 gm. Al_2O_3	10·8	10·60	—0·20	—1·85
III				
Calcium carbonate ($CaCO_3$) equivalent to—				
1. 0·02 gm. CaO	10·8	10·70	—0·10	—0·93
2. 0·04 gm. CaO	10·8	10·60	—0·20	—1·85
3. 0·08 gm. CaO	10·8	10·60	—0·20	—1·85
4. 0·10 gm. CaO	10·8	10·90	+0·10	+0·93
IV				
Magnesium sulphate ($MgSO_4.7H_2O$) equivalent to—				
1. 0·02 gm. MgO	10·8	10·70	—0·10	—0·93
2. 0·04 gm. MgO	10·8	10·70	—0·10	—0·93
3. 0·08 gm. MgO	10·8	10·90	+0·10	+0·93
4. 1·00 gm. MgO	10·8	11·00	+0·20	+1·85

Experiment 5

This was conducted to estimate variable amounts of potassium oxide in the presence of different groups of bases when present in normal quantities as in soils. The experimental procedure adopted for the determination of potash in these complex mixtures is as follows :—

The mixture containing potash is evaporated to dryness on a water bath ; the residue is then extracted with hot water and filtered. About 5 to 10 c. c. of 10 per cent citric acid is then added to the filtrate depending on the amounts of iron and alumina present therein and the resultant mixture neutralised with sodium carbonate of similar strength. The neutralised solution is further rendered slightly acidic by the addition of strong acetic acid. Citric acid is added to avoid the precipitation of basic acetates, especially iron, by the free acetic acid in the reagent itself when the mixture is concentrated on a water bath. The potash solution is then evaporated to a pasty consistency, stirring repeatedly, especially towards the end, to break the crystalline mass adhering to the sides of the dish. After cooling, the precipitate is treated with 5 to 10 c. c. of 10 per cent acetic acid saturated with sodium cobaltinitrite precipitate of potassium washed with this solution and then filtered over a Gooch with an asbestos felt with a disc of Whatman No. 40 filter paper on it. For the preparation of the filter the asbestos was first digested with

potassium permanganate and subsequently with an excess of oxalic acid. The digested asbestos is further washed with distilled water till free from acid and then used. The potash in the precipitate is then determined volumetrically as in Drushel's [1907, 1908] method, outlined already in the beginning of the paper. From the values recorded in Table VI, it may be seen that there is practically no difference between the theoretical and estimated figures, considering the small quantities of potash handled. The percentage error works out to less than 2 in all cases.

TABLE VI

Effect of different groups of metallic radicals on the volumetric estimation of K_2O by the cobaltinitrite method

Mixture No.	Metallic salts used	K_2O added theoretical (mg.)	K_2O recovered, average of four determinations (mg.)	Deviation of the estimated value from the theoretical (mg.) + or —	Per cent error of K_2O recovered + or —
I	Ferrous sulphate equivalent to 0·3 gm. Fe_2O_3	5·40	5·43	+0·03	+0·56
		10·80	10·70	—0·10	—0·93
		21·60	22·00	+0·40	+1·85
		27·00	26·70	—0·30	—1·11
II	Ferrous sulphate equivalent to 0·3 gm. Fe_2O_3 + Aluminium sulphate equivalent 0·3 gm. Al_2O_3	5·40	5·48	+0·08	+1·48
		10·80	10·60	—0·20	—1·85
		21·60	21·20	—0·40	—1·85
		27·00	26·50	—0·50	—1·85
III	Ferrous sulphate equivalent to 0·3 gm. Fe_2O_3 + Aluminium sulphate equivalent to 0·3 gm. Al_2O_3 + Tricalcium phosphate equivalent to 0·02 gm. CaO	5·40	5·48	+0·02	+0·36
		10·80	10·70	—0·10	—0·93
		21·60	21·30	—0·30	—1·39
		27·00	26·80	—0·20	—0·74
IV	Ferrous sulphate equivalent to 0·3 gm. Fe_2O_3 + Aluminium sulphate equivalent to 0·3 gm. Al_2O_3 + Tricalcium phosphate equivalent to 0·02 gm. CaO + Magnesium sulphate equivalent to 0·02 gm. MgO	5·40	5·35	—0·05	—0·93
		10·80	10·80	*Nil*	*Nil*
		21·60	21·30	—0·30	—1·85
		27·00	26·70	—0·30	—1·11

Experiment 6

Application of the method to soils.—A convenient aliquot portion (50/500 c.c.) of the hydrochloric acid extract of about 20 grms. ignited soil, prepared in the usual manner is evaporated to dryness on a water bath and the residue extracted with hot water and filtered. The resulting solution is then treated with 5 to 10 c. c. of 10 per cent citric acid, depending on iron and other bases present and the whole rendered slightly alkaline with 10 per cent sodium carbonate. The excess carbonate is neutralised with acetic acid till the mixture is distinctly acidic. The potash is then precipitated and determined exactly on the same lines as for the artificial mixtures, using *N*/20 oxalic acid and permanganate for titration. For the experimental verification of this method, ten different soils representing important types from the various parts of the Presidency were taken, namely (1) black cotton soil, (2) garden soil, (3) paddy soil, (4) estate soil rich in organic matter, (5) orchard soil and (6) red soil. Potash is determined in hydrochloric acid

extracts of these soils both directly and after the removal of the bases by sodium carbonate. The results obtained by these procedures were compared with those obtained by the usual platinichloride method, Lindo Gladding modification. From Table VII it may be seen that the results from all these procedures agree with one another more or less very closely.

TABLE VII

Potash estimation by the cobaltinitrite (volumetric) and platinichloride methods in HCl extracts of soils

Types of soils used	K_2O by the cobaltinitrite volumetric procedure (average of two determinations)		K_2O by the platinichloride method—Lindo Gladding—average of two determinations (mg.)	Per cent recovery of K_2O by the cobaltinitrite procedure over platinichloride method	
	Without removing the bases (mg.)	After the removal of bases (mg.)		In presence of bases	In absence of bases
I. Black cotton soil—No. (1)	14·46	14·40	14·50	99·72	99·31
„ „ „ „ (2)	12·32	12·36	12·40	99·35	99·68
II. Garden soil—No. (1) .	18·70	18·80	18·87	99·08	99·63
„ „ „ (2) .	17·40	17·38	17·45	99·49	99·60
III. Paddy soil—No. (1) . .	14·08	14·18	14·10	99·84	100·56
„ „ „ (2) . .	12·18	12·10	12·12	100·49	99·83
IV. Red soil—No. (1) . .	7·60	7·58	7·62	99·74	99·47
„ „ (2) . .	10·06	10·12	10·10	99·60	100·20
V. Estate soil . . .	24·12	24·30	24·20	99·68	100·40
VI. Orchard soil . . .	28·10	28·18	28·21	99·61	99·89

NOTE.—Figures represent potash in 100/500 c. c. of the HCl extract of about 20 grms. of ignited soil for each experiment. Two different soils were used to represent each of the types from I to IV except in the case of V and VI.

The method recommended above for hydrochloric acid extracts of soils is a deviation from those adopted by many investigators in the field in certain respects and attempt has been made to simplify the procedure. The removal of the bases by sodium carbonate before precipitation recommended by Bowser [1909] has been found unnecessary, provided the citric acid procedure is resorted to. The introduction of citric acid in the medium does not in any way seem to interfere with the precipitation of potash and its chief function is to keep iron, alumina, phosphates, etc. in solution.

The aliquot taken for the determination of potash in the above experiments are 100/500 c. c. portions of the hydrochloric acid extracts of about 20 grms. ignited soil and these have been found very convenient to handle. To test the soundness of the method still further known quantities of potash in the form of potassium sulphate were added to 100 c. c. portions of the hydrochloric acid extracts of the typical soils and the total potash estimated volumetrically without removing the bases. A glance at Table VIII will show that practically almost all the added quantities have been recovered, thereby confirming the reliability of the method.

TABLE VIII

Recovery of added potash from HCl extracts of soils by the cobaltinitrite (volumetric) method

Soils used	K_2O in soil extract, chloroplatinate method (mg.)	K_2O added to soil extract (mg.)	Total K_2O in soil extract (mg.)	Total K_2O recovered by the cobaltinitrite method, average of two determinations (mg.)	Recovery of added K_2O by the cobaltinitrite method (mg.)	Per cent K_2O recovered over theoretical
Black cotton soil . .	14·50	5·40	19·90	19·88	5·38	99·63
Garden soil . . .	18·87	5·40	24·27	24·24	5·37	99·44
Paddy soil . . .	14·10	5·40	19·50	19·55	5·45	100·93
Estate soil	24·20	5·40	29·60	29·62	5·42	100·37
Red soil	10·10	5·40	15·50	15·51	5·41	100·20
Orchard soil . . .	28·21	5·40	33·61	33·56	5·35	99·08

In the case of citric acid (1 per cent) extracts of soils, a convenient aliquot (containing 5 to 25 mg. of potash) from each extract is evaporated and ignited at a low temperature. The residue is dissolved in dilute hydrochloric acid, evaporated to dryness, gently ignited and extracted with 50 to 100 c. c. of boiling water. The filtered extract is rendered acidic with acetic acid and potash determined as in the case of pure salts. Known amounts of potash in the form of potassium sulphate were also added to citric acid extracts of soils before evaporation and ignition and the recovery of added potash effected almost completely. The results obtained are presented in Table IX.

TABLE IX

Recovery of potash from citric acid (1 per cent) extracts of soils by the cobaltinitrite (volumetric) method

Soils used	K_2O in soil extract, chloroplatinate method (mg.)	K_2O added to soil extract (mg.)	Total K_2O in soil extract (mg.)	Total K_2O recovered by the cobaltinitrite method, average of two determinations (mg.)	Recovery of added K_2O by the cobaltinitrite method (mg.)	Per cent K_2O recovered over theoretical
1. Black cotton soil . .	8·63	3·70	12·33	12·29	3·66	98·92
2. Garden soil . . .	9·38	5·20	14·58	14·52	5·14	98·85
3. Paddy soil . . .	5·76	7·50	13·26	13·18	7·42	98·94
4. Estate soil . . .	10·42	5·40	15·82	15·75	5·33	98·70
5. Orchard soil . . .	5·84	8·10	13·94	13·89	8·05	99·38
6. Red soil . . .	4·32	10·80	15·12	15·08	10·76	99·63

NOTE.—The recovery of potash is almost quantitative in each case.

SUMMARY

1. The cobaltinitrite method of estimating potash, both volumetrically and gravimetrically, has been tested with reference to simple salts, artificial mixtures of various bases, and acids, as well as hydrochloric acid and citric acid extracts of soils. It has been found reliable and quick within the ranges tried—3 to 53 mg. of potassium oxide. The volumetric procedure especially may be of immense use in determining small quantities of potash, even below 1 mg. as are usually met with in citric acid and ammonium chloride extracts of soils.

2. The method is least expensive and can be applied in the presence of sulphates, phosphates and other bases and in these respects it has distinct advantages over the platinichloride and perchlorate methods.

3. A simple modified volumetric procedure of the method for the direct estimation of potash without removing the bases in the hydrochloric acid extracts of soils has been outlined.

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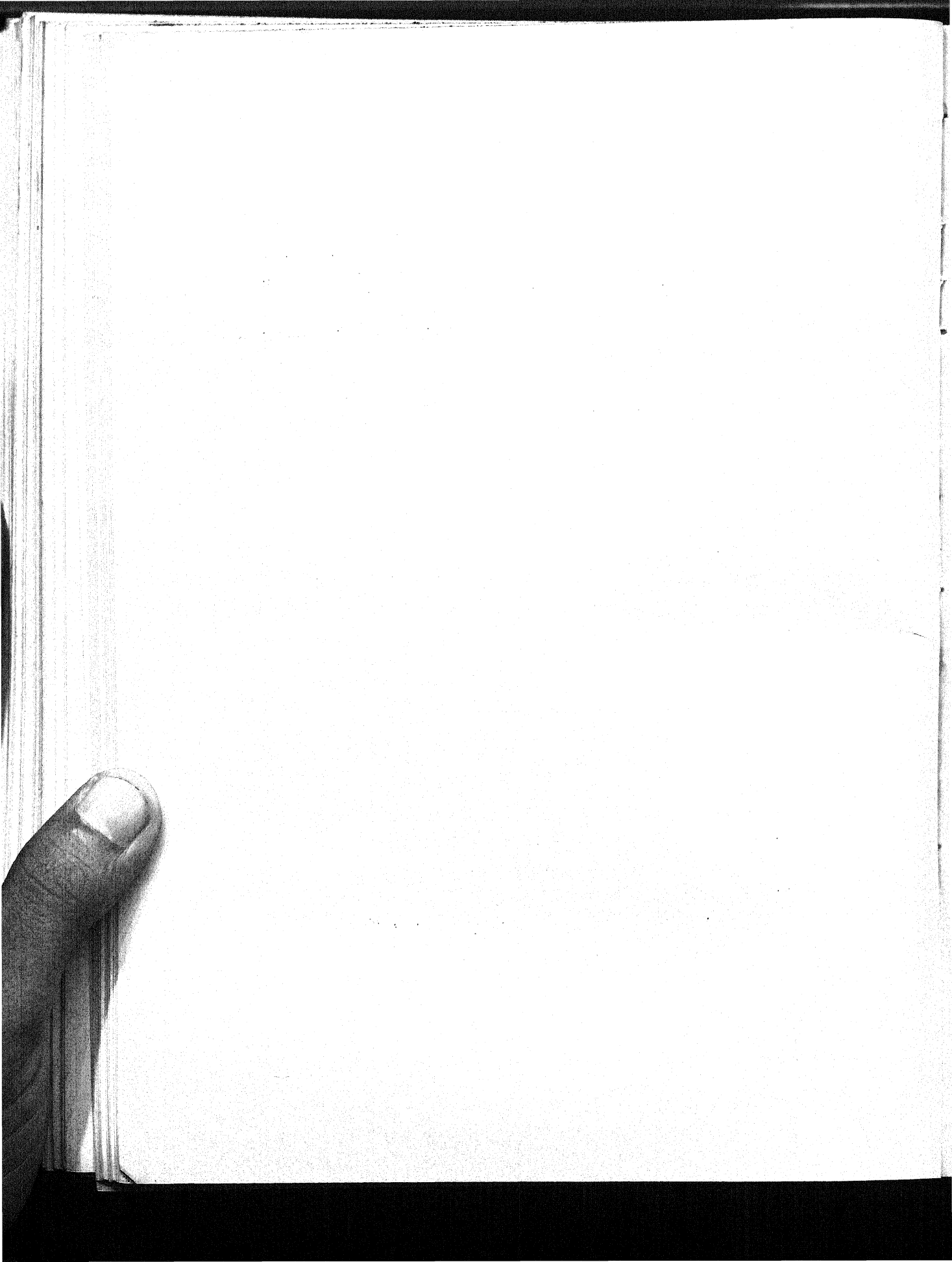

ABSTRACTS

A New Species of Azotobacter. ROBERT L. STARKEY and P. K. DE (*Soil Sci.*, VOL. 47, NO. 4, 1938)

DURING the course of an investigation of nitrogen fixation in rice soils of of India several cultures of nitrogen-fixing bacteria were isolated. From two acid soils cultures were obtained which were alike among themselves but different from any previously described species of *Azotobacter*. In consideration of these differences the organism was described as a new species, and named *Azotobacter indicum*.

The two soils were more acid than those from which *Azotobacter* is commonly recovered: Dacca soil of *p*H 4·9 and soil from Insein, Burma of *p*H 5·2. The organism was isolated on silica gel plates containing sucrose. Pure cultures grew well in nitrogen-free media containing sucrose and glucose. Dextrin was not utilised. Although development was very slow upon mannite, considerable amounts of nitrogen were fixed in a solution medium containing this material during prolonged incubation. In the presence of $CaCO_3$ growth was very much depressed.

In comparison with other species of *Azotobacter* the new organism grew very slowly; visible growth could seldom be detected before three to four days at the incubation temperature of 30°C. The surface colonies were uniformly turbid and starchy in appearance, and were very much raised, round and dome-shaped. The colonies fused into mucoid masses when they was not well separated from one another. Development on solid media was characterised by the production of a profusion of slime which gave the cell material a very mucoid, gummy and tenacious consistency. It could be drawn out in long threads with a needle and was somewhat elastic. The material was at first colourless but became buff to reddish brown during incubation for three weeks or longer.

The organism failed to grow on nutrient agar, or on peptone agar but developed slowly upon a medium containing both peptone and glucose and in other media containing carbohydrate in addition to nitrogenous organic materials.

The cells were Gram-negative and motile and measured 0·5—1·2 × 1·7—2·7μ. They differed principally from all other species of *Azotobacter* in that there were large fat globules in the cells. Generally there was one large globule at each end of the rod-shaped cells, but cells were also seen with only one terminal globule. A large portion of the cell, frequently more than one-half of the total volume, was occupied by the fatty material. The cells changed very slightly in morphology during prolonged incubation in

nitrogen-free carbohydrate media but became abnormal in media containing certain nitrogenous materials. In the latter case some of the cells were much larger, whereas others were swollen, elongated, unsymmetrical, budded, or yeastlike. No cysts or spores were detected under any condition.

The amounts of nitrogen fixed by *Az. indicum* were comparable to those fixed by other species of *Azotobacter*. On silica gel plates, between 7·0 and 9·8 mg. were fixed per plate containing 1·0 grm. of sucrose. In later tests there was an average of 6·0 mg. per plate with 1·5 grm. of glucose. Between 3·1 and 9·0 mg. of nitrogen were fixed per flask of solution medium containing 1·5 grm. of glucose or sucrose where $CaCO_3$ was added. In later experiments without $CaCO_3$ there was much greater fixation; between 13·5 and 19·4 mg. of nitrogen were fixed in solution media with glucose or sucrose and 9·9 mg. with mannitol.

Development in solution media was characterised by strong turbidity, by the accumulation of considerable sediment which increases as the incubation period was prolonged, and by the formation of a ring of slimy material on the wall of the flask at the surface of the liquid. There was no pellicle and practically no evidence of pigmentation. (*Authors' abstract*)

Sodium Ferrocyanide as Dispersing Agent in Mechanical Analysis of Soils.

BARADANANDA CHATTERJEE and BASANTA KUMAR DAS

(*Soil Research*, Vol. 6, No. 4/5, page 227, 1939)

THE pre-treatment of soil with acid and the final dispersion in a highly alkaline medium in the mechanical analysis of soils are open to criticism. A procedure has been adopted in which these two undesirable treatments are omitted. The soil is treated with 6 per cent H_2O_2 for removing the organic matter. It is then leached with about 500 c.c. of normal NaCl, then twice with 100 c.c. 0·01*N*—NaCl and finally with distilled water. The final dispersion is made in 0·002*M* $Na_4Fe(CN)_6$ by shaking and the suspension is subjected to pipette sampling. It has been found that—

(*i*) dispersion by $Na_4Fe(CN)_6$ is as efficient as in the International-soda method,

(*ii*) final dispersion is affected at much lower *p*H ; and

(*iii*) even laterite soils can be efficiently dispersed by $Na_4Fe(CN)_6$ at a *p*H as low as 6·47.

The addition of increasing amounts of NaOH to ferrocyanide, though increases the *p*H of the suspension, does not materially increase it dispersing action on the clay. The efficient dispersion of soils by $Na_4Fe(CN)_6$ has been ascribed to the adsorption of polyvalent anions by the negatively charged oil colloids. (*Authors' abstract*)

NOTE

NOTICE NO. 2 OF 1939

PLANT Quarantine Regulations and Import Restrictions relating to the following countries have been received. Those interested are advised to apply to the Secretary, Imperial Council of Agricultural Research.

United States Department of Agriculture, Bureau of Entomology and Plant Quarantine—

1. White fringed beetle quarantine (No. 72)
2. Information for the press—Revised pink boll-worm quarantine and restrictions
3. Service and regulatory announcements, October-December 1938

Summaries of the Plant Quarantine and Import Restrictions, issued by the United States Department of Agriculture, Bureau of Entomology and Plant Quarantine of :—

4. Albania
5. British Gold Coast Colony
6. British Colony of Ceylon
7. Kingdom of Italy
8. Commonwealth of Australia (Supplement No. 2)
9. Chosen (Korea)
10. Kingdom of Yugoslavia (Supplement No. 4)
11. Iran

Notice regarding the Regulations for the prevention of disease amongst plants.

REVIEWS

The Rape of the Earth : A World Survey of Soil Erosion. By G. V. JACKS and R. O. WHYTE. Faber and Faber. 21*s*.

JUDGING from the large amount of concrete evidence produced in many other countries of the drier tropics and semi-tropics, soil erosion must be accepted as a serious fact and not a mere subject for dialectics and debate. It is not confined to the United States of America nor is it another American newspaper man's publicity stunt, although American papers continue to give this subject good front page value as news. It is proved by this book, if proof were needed, that almost every country of the world is suffering to some extent from preventable soil losses and that India is undoubtedly one of the worst sufferers. The authors who are both recognised authorities each in his own field, Jacks as a soil chemist and Whyte as a grassland ecologist, consider that a national catastrophe due to soil erosion and its dreadful consequences is perhaps more imminent in South Africa than in any other country. General Smuts, quoted in this book, has stated that " erosion is the biggest problem confronting the country, bigger than any politics ". In spite of the publicity focussed upon this subject in America the authors consider that the African continent must now take the foreground for erosion " news value ". In the United States of America the federal and states governments are teaching the farmers to grapple with local phases of the erosion problem and this work is now going forward on an immense scale and in an orderly and organised fashion, whereas in Africa the need for soil conservation measures is even more urgent but the problem cuts across the social customs of diverse races, the habits of life of ignorant and suspicious pastoral tribes, conflicting interests of innumerable forms of government, and demands of white and black populations, sometimes mixed, sometimes segregated.

But where does India stand between these two extremes and how can Indian land-use practices be improved in the light of this newly acquired knowledge in these other continents ? This is what the authors say of India : " Over much of India the people have accepted a slowly deteriorating environment as part of the scheme of things ; the vast majority is probably unaware that erosion is occurring." This is unfortunately a painfully true statement, and makes the introduction of control measures a much more difficult task than in countries where every farmer is able to understand the danger and willing to take his share in erosion control measures.

Of the two great causes of erosion damage, grazing is given considerably more space than field cultivation for the world as a whole and for India in particular, but this does not necessarily reflect the importance of their relative contributions. The actual steps necessary to obtain control of soil erosion are already known, but it is quite another matter to put them into effect against

the opposition of conservative and suspicious villagers. The social repercussions of attempts at control of grazing are usually much more serious than in the more straightforward issues of field crops and plough-lands, and as due weight is given to this very important side of the work, the control of grazing on wild lands is dealt with in considerable detail. The authors agree that constructive work can best be done on a basis of self-help and free labour contributions, because provincial governments can hardly be expected to undertake this for each of the thousands of villages which require attention. The two main heads for village activity to be dealt with, preferably through their own Co-operative Society or Panchayat, are : (1) to develop fodder resources for local live-stock, and (2) to improve wild lands so as to conserve moisture and reduce run-off. Common and useful lines of attack are :—the replacement of grazing by grass-cutting ; rotational closure of grazing land ; partition of *shamlat* or common waste land ; intensive improvement of natural grasslands by means of " gully plugging " with small checkdams of any material which may be locally available ; contour ridging and trenching to encourage better grass growth on ground closed to grazing ; determination of the correct grazing incidence for local types of bush and grass ; development of tree fodder supply with controlled rotational lopping ; encouragement of green fodder crops and silage ; and most important of all, the reduction of herds by a campaign for fewer and better cattle.

To most of our readers, however, the main interest is probably in control measures as applied to field crops, and it is interesting to read these experts' opinions. Great emphasis is laid on the need for imitating the American drive for soil conservation measures in plough-land, but they point out the dangers of slavish copying of unsuitable or untried methods. Much can be suggested from an empirical knowledge of control measures used elsewhere, but the great need in India at the moment is to work out a local technique for the many variations in crops and types of soil and slope, particularly as affecting local recommendations for contour ridging (*watt-bandi*) practice. The local method of bund-building varies from place to place according to the caste of the cultivator. For instance a Jat village can be trusted to have a large percentage of its fields properly *watted* while neighbouring Rajputs and Gujars may not even have started. When the use of the ox-drawn wooden scoop or *karah* is universal in the rest of the Punjab why is it almost entirely unknown in a comparatively central area such as Gurdaspur ?

At the moment there is considerable enthusiasm amongst civil officers for advocating *watt-bandi* without sufficient knowledge of the serious effect upon certain crops through the building of large field *watts* or bunds. Cotton, maize and *bajra*, for instance, are liable to suffer badly from sun-scorch if they stand for two or three days in water which has been caught behind a two-feet high bund. The reviewer recommends as a safer alternative for such crops to multiply the number of parallel bunds and make them much smaller and closer together, say six inches high and eight to ten feet apart, so that the water caught behind each will have seeped away in a few hours. In the case of light *barani* soils used for winter wheat and left as a bare fallow in summer, there is

no danger whatever in building larger *bunds* further apart ; the urgent point here is to get the fallow ploughed before the monsoon breaks and to keep it broken by two or three subsequent monsoon ploughings to ensure good seepage. The book under review issues repeated warnings on the subject of bare fallow, but so far India has not found any panacea for the sheet erosion from fallow fields like the *Lespedeza* now used in some variety or other for sowing on fallow in a great many parts of the United States of America. Here lies a brilliant opportunity for the Provincial Agriculture Departments to work out their own and the villagers' salvation by finding a cheap and handy leguminous or grass plant which could be sown on fallow to protect the soil from the onslaught of monsoon storms ; it should also produce some fodder and improve the soil when ploughed in as a green manure.

It is interesting to read that in almost every country it is the method of land tenure that forms the worst stumbling-block to the introduction of control in plough-lands. Tenant farmers never seem to get the best out of the land, because it tends to deteriorate when it is worked without considering the long-term consequences. Short leases prevent the use of soil-improving crops or of a rotation of crops. It would appear therefore that improvement lies in the hands of the Land Revenue staff because the *patwari*, guided by the revenue assistant, can bring some weight to bear on such problems. The existing revenue staff is, however, fully occupied with its routine work and can hardly be expected to undertake extra tasks. Another possible alternative is to enlarge the scope of the special co-operative staff which now handles the consolidation of holdings, and make it responsible for the partition of *shamlat* and the encouragement of *watt-bandi*. In many of the foothill villages consolidation is of practical importance only in the intensively cultivated home-fields nearest to the dwellings, *watt-bandi* is badly needed in all the rest of the out-field land, while partition may be desirable in the *shamlat* to encourage the creation of hay-fields or the reclamation of gullied waste.

A part of the authors' soil conservation recommendations which has been sadly neglected so far in India is tree shelter-belts. A great deal of shelter-belt planting was undertaken in Russia in the more arid parts of the wheat-growing steppe some 60 years ago and the results have been so satisfactory that the Soviet is now carrying out a programme for establishing nearly 900,000 acres of shelter-belt in their current Five Years' Plan. The United States of America is also forging ahead with its $1\frac{1}{4}$ million acres of planting in the Great Plains shelter-belt project. In the Californian citrus-growing districts which are similar in many respects to the irrigated fruit farms of the Punjab, shelter-belts are considered absolutely essential ; 10 per cent of the area of each Californian garden is usually kept under wind-break trees and as much as 40 rupees per orchard acre is budgetted for wind-break maintenance. With the extremes of heat, drought and cold which the Punjab experiences it is surprising that so little attention has been paid to this question, and throughout India there is a great need for the closer union and co-ordination of forestry with agriculture in dealing with the everyday problems of the cultivator. This book should be in the hands of everyone who is in any way responsible for the growing of plants and the produce of the soil. [R. M. G.]

Fruit Juices and Related Products. (*Technical Communication No. 11 of the Imperial Bureau of Horticulture and Plantation Crops, East Malling, Kent, England*). By V. L. S. CHARLEY, and T. H. J. HARRISON, 1939. Figures 49. Bibliography 118. Price 5*s*.

WHETHER we have suddenly developed sense, or whether the old slogan "an apple a day keeps the doctor away" has at last struck home, is immaterial. The fact remains that we do eat more fruit and that more fruit is being produced. Market quality standards have become higher and growers are now faced with the problem of how to dispose satisfactorily of fruit which does not come up to the accepted standards of size or colour, though otherwise perfectly good. Among processes offering a solution, that of unfermented juice production has long commended itself to fruit producers in Europe and the U. S. A.

Hitherto, though short articles on different aspects of processing have been available since 1914 in the annual reports of the Long Ashton Research Station, lack of precise information on the exact technique employed has checked progress in England.

It has remained for Charley to give under one cover a complete detailed account of the manufacture of apple juice based on his knowledge of the latest foreign methods and on his own continued investigations at Long Ashton.

In the present bulletin he deals with the manufacturing process in detail, starting with the selection of the fruit and taking us through every stage in turn up to the disposal of the final product in bottle, can or other type of dispenser. He does not confine his remarks to any one process but gives particulars of different methods found satisfactory and discusses their merits.

Further he deals more briefly with such other fruit products as grape, citrus and soft fruit juices, cider, fruit wines, etc., with the concentration of fruit juices by hot processing, by freezing and by spray drying, and with the disposal of pomace.

He describes methods of analysing juice for the determination of specific gravity, total acidity, tannin, alcohol and sulphur dioxide.

Finally he discusses the suitability of particular metals and alloys for the construction of the requisite apparatus, the clear illustrations of which add greatly to the usefulness of the publication.

But the problems of juice production do not, unfortunately, begin and end with its manufacture, and the economic and nutritional aspects are equally important. We are, therefore, grateful to Harrison for his concise and adequate treatment of them in the first twenty pages.

He shows that a permanent fruit juice industry on a large scale can only be developed where large supplies of raw material are available each year at a reasonable price. The cost of manufacture is not small, and if sales are to grow it is essential that retail prices should be reasonably low so as to compete with other drinks. This means that distribution costs must be low and that the industry must depend essentially on its home market. Here regulations are necessary to protect it from competition with synthetic products.

The nutritive and therapeutic qualities of fruit juices are discussed at sufficient length to show their great value, not only to children and invalids and for the treatment of gastric troubles, but also in the diet of the normal healthy adult.

A short glossary is included for the use of the less expert, and this together with the comprehensive index should make the information readily accessible to all.

The Action and Use of Colchicine in the Production of Polyploid Plants. By J. L. FYFE. Issued by the Imperial Bureau of Plant Breeding and Genetics (for Crops other than Herbage), Cambridge. Pp. 10. Price 1*s*.

THE discovery of the action of the drug colchicine on nuclear division, making it the most reliable agent yet used in the production of polyploid plants, has excited great interest even in laymen and is of importance to those working on the cytology or genetics of plants.

The Imperial Bureau of Plant Breeding and Genetics, Cambridge, has issued a bulletin on the subject, summarising the already fairly extensive literature. This bulletin begins by explaining the chromosome doubling; next a detailed account of the action of colchicine on mitosis and meiosis is given, followed by summaries of the results obtained from the use of colchicine for producing polyploid plants. The action and use of acenaphthene, which the Russian workers have shown to be a similar agent to colchicine are also described. Particular attention has been paid throughout to treatments and dosages and some recommendations are given which should be helpful to those contemplating the production of polyploids. All the literature appearing up to the end of January 1939 is included and listed in a bibliography of 38 references.

Bibliography of Baking Quality Tests (Supplement). Issued by the Imperial Bureau of Plant Breeding and Genetics (for Crops other than Herbage), Cambridge. Pp. 10. Price 1*s*. 6*d*.

THE valuable "Bibliography of Baking Quality Tests" issued by the Imperial Bureau of Plant Breeding and Genetics in 1934 has been brought up to date by the publication of a supplement which contains titles of papers from 1933 to 1938 inclusive.

Some of the papers noted have been abstracted in " Plant Breeding Abstracts " but many, because of their specialist nature, have not been reviewed but will thus become available to workers on the subject.

A note of volume and abstract number in the margin indicates where an abstract may be found in " Plant Breeding Abstracts ". The titles are arranged alphabetically according to authors and, given sufficient demand, further supplement are promised from time to time.

ORIGINAL ARTICLES

FURTHER STUDIES ON THE INHERITANCE OF LEAF SHAPE IN ASIATIC *GOSSYPIUMS*

BY

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(Received for publication on 28th March 1939)

(With one text-figure)

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Introduction

In a previous paper by the senior author [Hutchinson, 1934] on the inheritance of leaf shape in Asiatic cottons, a theory of the organization of the gene was put forward to account for the relationships between members of the leaf shape multiple allelomorph series. According to the theory, $\mathbf{L}^{L}$ (laciniated) carries four episomes on a single protosome, and **L** (narrow) carries one only. Intermediate allelomorphs carrying two or three episomes should be possible, and a search was made for them in the extensive collection of Indian types of *G. arboreum* var. *neglectum* (classification according to Hutchinson and Ghose [1937]) maintained at Indore. A new allelomorph was found in a strain known as 1090 obtained from Nagpur, Central Provinces. It will be given the symbol $\mathbf{L}^{N}$.

Data

1090 is a narrow-leaved type of *G. arboreum* var. *neglectum*. It has a deeper cut sinus, giving a higher index A than types carrying the **L** allelomorph, but about the same lobe width (and index B). (For definitions of the leaf indices used, see Hutchinson [1934]). 1090 resembles Burma laciniated and differs from strains carrying **L** in having no accessory lobes in the

sinuses between the main lobes (Fig. 1). Below are given leaf indices (at Indore) of typical strains carrying L^L, L and l for comparison with 1090.

Strain	Index A	Index B	Allelomorph
Burma laciniated	11·1	6·6	L^L
1090	6·8	3·8	L
Cawnpore White	3·5	4·0	L
Kokati narrow	3·3	3·9	L
1304	2·2	2·4	l
Kokati broad	2·2	2·5	l
Malvi 9	2·1	2·3	l

An outline of a 1090 leaf is given in Fig. 1.

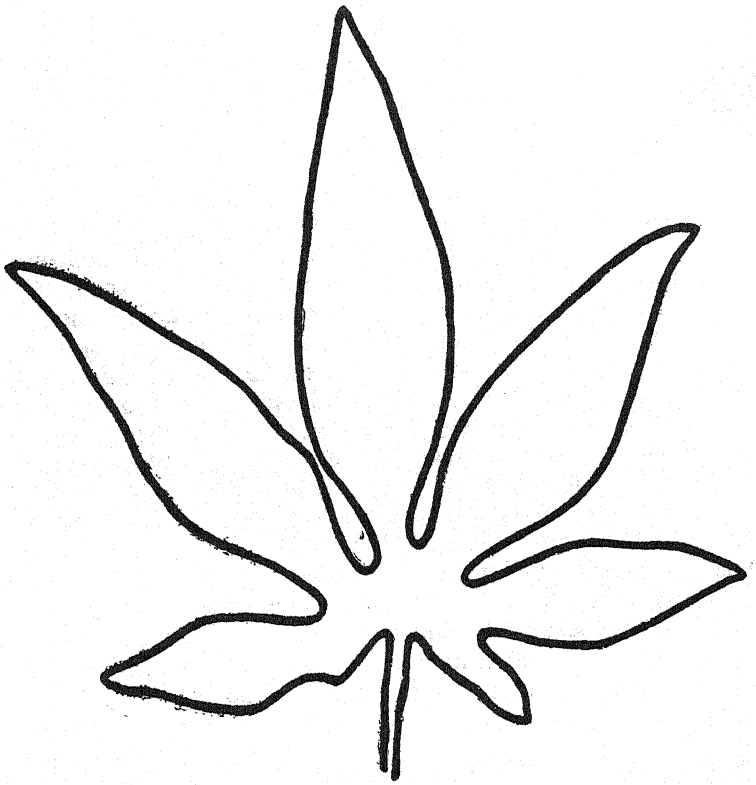

FIG. 1. Outline of a 1090 leaf

1090 was crossed with Cawnpore White and 1304, two of the strains used in Hutchinson's [1934] earlier investigation on leaf shape, and also with narrow and broad-leaved strains isolated from a brown-linted form of *G. arboreum* var. *neglectum* known as 'Kokati'. Small F_1 families were grown and the ranges in leaf index are given below.

F_1	Index A	Index B
1090 × C. W.	3·9–5·8	3·7–4·3
1090 × Kokati narrow	4·1–6·5	3·3–4·6
1090 × 1304	3·6–5·2	2·8–3·2
1090 × Kokati broad	4·5–5·2	3·1–3·4

In the F_1 accessory lobes occurred only rarely.

F_2's and back-crosses to either Cawnpore White or Malvi 9 were grown. Correlation tables of index A and index B are given in Tables I–IV for F_2's and VI-IX for back-crosses. The 1090 × C. W. and 1090 × 1304 F_2's and back-crosses were grown in 1935-36, and plants were classed as either with or without accessory lobes, those having occasional accessory lobes, as in the F_1, being classed as without. The Kokati crosses were grown in 1936-37, and were divided into three classes, with accessory lobes, without, and with occasional accessory lobes. In the tables, totals are given for accessory lobes present (+), absent (0), and occasional (?) for both index A and index B. The leaf index of narrow leaves (**L**, $\mathbf{L^N}$ and $\mathbf{L^L}$) varies considerably with the season and the stage of development at which measurements are made. There are consequently considerable differences in the mean indices of $\mathbf{L^N}$ and **L** classes in different years and to some extent between families grown in the same year. Accurate comparisons of indices are therefore only possible between classes in the same family, unless the material has been grown under specially controlled conditions.

In F_2's and back-crosses of crosses with **L** strains (Cawnpore White and Kokati narrow) the correlation between index A and index B was much lower than in $\mathbf{L} \times \mathbf{L^L}$ crosses (+ 0·40 and + 0·17 against + 0·86). Segregation was much more pronounced for index A than for index B, and plants with accessory lobes had a low index A, while those without, or with only occasional accessory lobes had a high index A. Overlapping occurred as would be expected, but the difference in index A between plants with accessory lobes and those with few or none is large and clear. In index B, as would be expected from the similarity of the parents, there was little evidence of segregation, and only a small difference between the accessory lobe classes. Segregation in index A was not sharp enough to allow of the separation of the two classes but it seemed probable that a close approach to the correct

classification would be obtained by taking the 'no accessory lobes' *plus* 'doubtful' as representing the 1090 and heterozygote classes, and the 'accessory lobes present' as the **L** class. Summaries are given below.

Cross	Accessory lobes			Total
	Present	Doubtful	Absent	
1090 × C. W.—F_2	33	..	97	130
(1090 × C. W) C. W.	8	..	9	17
1090 × Kokati narrow—F_2. . .	106	66	268	440
(1090 × Kokati narrow) M9 . .	29	11	20	60

The ratios obtained agree closely with the expected 3 : 1 and 1 : 1.

The Kokati parent carried the lint colour gene linked with the leaf shape multiple allelomorph series. If 1090 carries an allele in the **L** series, in F_2 the 1090 leaf class should be predominantly white linted, and the Kokati leaf class predominantly brown linted. Frequency arrays of index A for brown and white linted classes separately in F_2 and back-cross to M9 are given at the bottom of Tables II and VII. It will be seen that the white-linted class had a higher index A on the average than the brown-linted class, showing that the segregation in index A was due to a member of the **L** series linked with lint colour.

F_3 progenies were grown from 58 F_2 plants of 1090 × C.W., and were classified by eye into narrow like 1090 and narrow like C. W., and for presence or absence of accessory lobes. Frequency arrays of index A and index B of parents of F_3 progenies are given in Table I. F_3 progenies were of three kinds, homozygous high index A, no accessory lobes (12 progenies), segregating for index A and accessory lobes (33 progenies), and homozygous low index A with accessory lobes (13 progenies). The proportions are close to the expected 1 : 2 : 1 ratio. The F_3 progenies were classified by eye, and though a satisactory approach to a 3 : 1 ratio was realized in segregating progenies, it cannot be regarded as being very accurate, and details are therefore omitted. Of the parents of F_2's, all which gave homozygous high index A had a mean index A of 6·98 and no accessory lobes. Heterozygous F_2 plants had a mean index A of 5·25. Out of 33 plants, 29 had no accessory lobes, and 4, with index A at 3·2, 3·9, 4·2 and 4·3 had accessory lobes. Of the plants which gave homozygous **L** progenies, one had an index A of 8·0 and no accessory lobes. It is difficult to account for this unless there was some error in labelling. The other 12 had a mean index A of 3·56. Nine of them had accessory lobes and three, with index A at 3·7, 4·5 and 4·6, had none. Mean index B was 4·0 for all three classes. In index A the heterozygote was almost exactly half way between the homozygotes, showing no dominance. The development of accessory lobes is shown to be a fairly accurate test of the genotype, classification into $\mathbf{L^NL^N} + \mathbf{L^NL}$ *v.* **LL** being correct in 88 per cent of cases. In any case the F_2 behaviour demonstrates single-factor inheritance conclusively.

In F_2's and back-crosses of crosses with l strains, index A and index B were positively correlated, and taking the two together, a satisfactory distinction can be made between the 1090 plus heterozygote class, and the l class. The plants were classified for accessory lobes and the $L^N L^N$ and L^N l classes had none or only a few doubtful cases. In the ll class about half the plants had them. Segregation was sharper in index A than index B, however, as would be expected, since the difference between the parents was much greater in index A than in index B. Segregation for lint colour occurred in the Kokati cross. The leaf shape and lint colour segregation are summarized below :—

Cross	Broad	Ll	Narrow and intermediate	Total
1090 × 1304—F_2	39	..	114	152
(1090 × 1304) C. W.	..	17	22	39
1090 × Kokati broad—F_2—				
Brown	52	..	132	255
White	4	..	67	
(1090 × Kokati broad) M9	14	..	23	37

Three of the plants measured for leaf shape produced no lint, so the lint colour—leaf shape totals do not exactly match the leaf shape tables. Crossing over per cent = 24·8 in F_2. The leaf-shape ratios closely approach the expected 3 : 1 and 1 : 1 and the crossover percentage is not far from Hutchinson's [1934] estimates in crosses involving the lint colour—leaf shape linkage. 1090, therefore, carries an allelomorph of the **L** series.

F_3's were grown from 37 F_2 plants of 1090 × 1304. Apart from one abnormal progeny (discussed below), they were of three kinds, 13 progenies from plants with index A from 4·4 to 8·6 and index B from 3·0 to 4·6 which gave 1090 type (L^N) only, 13 progenies from plants with index A from 3·4 to 8·4 and index B from 3·0 to 4·0 which segregated into approximately 3, 1090 type : 1 broad, and 10 progenies from plants with index A from 2·0 to 3·0 and index B from 2·2 to 3·0 which gave broads (l) only. The defect of heterozygotes from the expected 1 : 2 : 1 is satisfactorily accounted for by the fact that a larger proportion of plants with high and low indices than with intermediate indices was selected as parents of F_3.

One F_2 plant with index A 5·8 and index B 4·2 behaved abnormally in F_3. Instead of giving 1090 types and broads only, it gave 11 like 1090 : 5 narrow. Four of those like 1090 were grown to F_4. Two bred true and two segregated, giving 12 like 1090 : 14 narrow. Of four narrows, two gave narrows only and two gave 26 like 1090 : 19 narrow. The labelling was carefully checked, and there was no evidence of error.

It is recorded here as it seems likely that an **L** gene appeared by mutation in homozygous L^N.

1090 was crossed with the Burma laciniated type B L. The F_1 was intermediate. Leaf measurements were taken and lint colour recorded on an F_2 of 400 plants. A correlation table of index A and index B is given in Table V. The two indices were highly correlated. The 1090 type segregates were distinguishable with confidence in the field by their broader lobes and shallower sinuses. The field classification agreed well with the measurements. Inspection of Table V will show that a group of plants with comparatively low indices (modes 5·0 index A and 3·6 index B) is separated from the rest by an area of low frequency. Dividing at the line passing through the area of low frequency gives 302 laciniated : 98 narrow, or very close to 3 : 1. This division agrees well with the eye classification, only six plants judged to be laciniated falling in the narrow class by measurement (marked with* in Table V). The Burma laciniated parent was brown linted, so the linkage between brown-lint and leaf shape should result in a preponderance of brown-linted laciniated, and white-linted narrow plants. There were 247$L^L K$: 55$L^L k$: 61$L^N K$: 37$L^N k$ giving 36·5 per cent crossing over, and confirming the allelomorphism of L^L and L^N.

Discussion and summary

It is clear from the leaf shape of the new allelomorph that no such simple scheme as that proposed by Hutchinson [1934] will fit the facts. The original hypothesis was successful in predicting the existence of other allelomorphs, but the one found fails to fit into the series. Detailed discussion of the relationships between the allelomorphs can only be made when they have been transferred to a common genetic background and measured under strictly comparable conditions. Some preliminary conclusions can be drawn from a comparison of the leaf indices of the parental types given below :

	Index A	Index B	Allelomorph
Burma laciniated	11·1	6·6	L^L
1090	6·8	3·8	L^N
Kokati narrow	3·3	3·9	L
Kokati broad	2·2	2·5	l

Index A and index B do not run parallel, L^N having a much higher index A and about the same index B as L types. This is borne out by the absence of segregation in index B in $L^N \times L$ crosses. The hypothesis can be modified to embrace the new facts by supposing that sinus length and lobe width are controlled by independent portions of the gene.

REFERENCES

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TABLE I

Correlation between index A and index B in 1090×C. W.—F_2

		Index A																																						Totals		Parents of F_3 progenies	
		2·6	2·8	3·0	3·2	3·4	3·6	3·8	4·0	4·2	4·4	4·6	4·8	5·0	5·2	5·4	5·6	5·8	6·0	6·2	6·4	6·6	6·8	7·0	7·2	7·4	7·6	7·8	8·0	8·2	8·4	8·6	8·8	9·0	9·2	9·4	9·6	+	0	like 1090	het.	L
Index B	5·4	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...
	5·2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...
	5·0	...	...	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	1	1	...	...	...	...	1	...	...	...	...	...	...	...	...	1	1	4	...	3	1
	4·8	...	...	...	...	...	...	...	...	1	...	...	...	...	...	...	...	1	...	1	...	1	...	...	...	...	...	...	1	...	...	...	1	...	...	...	...	1	5	1	1	1
	4·6	...	...	...	...	...	...	...	1	1	...	...	...	...	...	...	...	...	2	2	...	1	...	...	1	...	...	...	...	1	...	...	...	...	...	...	...	2	7	1	1	...
	4·4	...	...	...	...	...	...	...	...	1	...	...	1	2	...	...	1	...	1	...	...	1	...	...	...	...	...	...	...	1	...	...	...	...	...	...	...	1	1	8	1	4	...
	4·2	...	1	...	...	...	...	...	...	...	1	3	...	1	...	2	...	1	...	...	3	...	2	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	3	12	2	3	3
	4·0	...	...	1	3	...	...	1	3	1	1	1	...	4	2	3	1	1	2	1	...	...	...	2	...	...	...	1	...	...	...	...	...	...	...	...	...	...	8	20	1	8	3
	3·8	1	...	3	1	...	2	...	2	1	1	4	1	2	...	...	...	3	...	1	...	2	...	...	1	...	1	...	...	...	2	...	...	...	...	...	...	...	10	18	4	7	3
	3·6	2	2	...	...	...	...	1	...	...	1	...	...	2	...	3	1	3	1	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	4	13	1	3	2
	3·4	...	...	...	...	1	...	1	...	2	2	...	1	1	1	...	...	1	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	3	8	1	3	...
Totsal	+	3	3	4	4	1	1	...	6	5	3	3	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	33	...			
	0	...	...	...	...	...	1	3	1	2	3	5	3	12	3	8	3	10	6	5	5	7	3	3	2	1	1	2	1	1	3	...	1	...	...	...	2	...	97			
Parents of F_3 progenies	like 1090	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	3	2	...	1	1	...	...	...	1	...	1	...	...	2	...	1	...	...	...	...	...	...	12			
	het.	...	...	...	...	1	1	...	1	2	3	2	2	5	2	3	2	...	1	1	1	2	2	1	...	...	...	1	...	...	...	...	...	...	...	...	...	...	4	29			
	L	1	1	2	2	...	...	1	2	...	1	2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	...	10	3			

TABLE II

Correlation between index A and index B in 1090 × Kokati narrow—F_2

		Index A																										Total		
		2·0	2·2	2·4	2·6	2·8	3·0	3·2	3·4	3·6	3·8	4·0	4·2	4·4	4·6	4·8	5·0	5·2	5·4	5·6	5·8	6·0	6·2	6·4	6·6	6·8	7·0	+	?	0
Index B	4·8	...	...	...	...	...	...	1	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	2	...	...
	4·6	...	...	...	...	...	1	1	1	1	1	...	1	2	...	...	1	...	...	...	...	...	...	...	...	...	...	5	2	2
	4·4	...	...	...	...	...	2	3	1	2	...	1	...	2	5	3	2	...	1	1	...	...	...	...	...	...	1	6	2	16
	4·2	...	...	...	...	4	2	3	1	3	4	3	4	8	6	5	4	...	2	1	...	1	1	...	...	...	...	9	12	31
	4·0	...	...	...	1	8	6	7	4	5	3	7	11	11	3	3	3	2	1	2	1	2	1	...	...	1	...	27	11	44
	3·8	...	...	...	2	6	4	4	4	7	9	12	17	10	10	5	9	4	4	1	3	2	1	...	...	...	...	16	20	78
	3·6	...	...	1	9	3	5	3	6	6	9	12	6	8	3	4	3	4	3	1	...	...	2	...	...	...	...	19	14	55
	3·4	...	...	1	6	2	4	1	7	2	4	3	2	2	5		...	...	...	1	...	...	...	...	...	...	...	13	3	27
	3·2	...	...	3	...	2	...	3	2	...	1	1	1	1	1	2	2	...	1	1	...	...	...	...	...	...	...	5	2	14
	3·0	1	...	1	...	1	1	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	4	...	1
Totals	+	1	...	6	15	24	18	19	11	3	4	3	...	...	1	...	...	...	...	1	...	...	...	...	...	...	...	106	...	...
	?	...	...	...	...	...	...	3	6	9	7	13	10	8	3	3	2	1	...	...	...	...	1	...	...	...	...	...	66	...
	0	...	...	...	3	2	7	4	11	14	20	23	32	36	29	22	22	9	12	7	4	5	4	...	...	1	1	...	...	268
	Brown	1	...	5	18	22	17	20	24	23	27	31	31	32	24	17	14	4	7	4	4	1	1	...	...	...	1	328	Brown	
	White	...	...	1	...	4	8	6	4	3	4	8	11	12	9	8	10	6	5	4	...	4	4	...	...	1	...	112	White	

TABLE III

Correlation between index A and index B in 1090×1304—F_2

| | | Index A | | |

TABLE IV

Correlation between index A and index B in 1090 × Kokati broad—F_2

Index B	Index A: 1·8	2·0	2·2	2·4	2·6	2·8	3·0	3·2	3·4	3·6	3·8	4·0	4·2	4·4	4·6	4·8	5·0	5·2	5·4	5·6	5·8	6·0	6·2	6·4	6·6	6·8	7·0	7·2	Totals +	Totals ?	Totals 0
·6	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	...	...	...	1
4·4	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
4·2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	2	3	...	3	1	1	1	...	...	...	...	...	11
4·0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	2	1	...	...	2	1	1	1	...	1	...	2	...	14
3·8	...	...	...	...	...	...	...	1	...	...	...	1	...	...	...	1	2	1	...	...	2	1	1	1	...	1	1	...	...	1	21
3·6	...	...	...	...	...	...	...	...	...	...	...	1	3	...	3	3	2	2	1	4	1	...	...	...	1	1	...	...	...	2	39
3·4	...	...	...	...	1	..	...	2	2	2	3	6	4	6	3	4	3	1	2	1	...	...	...	...	...	1	...	...	...	2	49
3·2	...	1	1	1	...	1	1	2	2	2	10	11	7	7	...	2	...	1	...	...	...	...	1	...	...	1	...	...	...	1	43
3·0	...	...	...	1	...	1	2	1	8	6	8	4	3	3	5	1	1	...	...	...	...	...	...	...	...	...	...	...	7	1	17
2·8	...	2	4	1	3	1	3	1	2	6	2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	16	...	7
2·6	2	4	8	6	...	...	1	1	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	12	...	7
2·4	7	5	4	...	1	..	1	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	6
2·2	1	2	1	2	..	..	...	...	..	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1
2·0	1	...	...	..	..	..	..	..	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	35	...	...
+	7	4	15	6	2	..	1	..	...	...	...	...	...	4	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	7	...
?	...	...	...	...	...	...	...	..	...	1	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..	216
0	4	10	3	5	3	3	7	9	14	15	22	24	17	12	11	11	8	5	5	7	3	4	3	3	2	4	...	2	...	..	

TABLE V

Correlation table of index A and index B in 1090×B. L.—F_2

Index B \ Index A	3·4	3·6	3·8	4·0	4·2	4·4	4·6	4·8	5·0	5·2	5·4	5·6	5·8	6·0	6·2	6·4	6·6	6·8	7·0	7·2	7·4	7·6	7·8	8·0	8·2	8·4	8·6	8·8	9·0	9·2	9·4	9·6	9·8	10·0	10·2	10·4	10·6	10·8	11·0	11·2	11·4	11·6	11·8	12·0	12·2	12·4	12·6	12·8	13·0	above 13·0	$L^N K$	$L^N k$	$L^L K$	$L^L k$
8·6	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	1	...
8·4	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...
8·2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	2	1	...	...	...	1	...	...	...	...	...	...	...	1	...	...	5	...
8·0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	1	1	1	...	...	...	...	...	...	...	...	...	...	...	...	...	2	2
7·8	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	1	...
7·6	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	1	...	...	...	1	2	...	...	1	...	...	4	...	...	9	1
7·4	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	...	...	...	...	1	...	...	...	...	1	2	1	...	...	...	...	...	...	2	...	...	7	1
7·2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	...	...	1	...	...	...	...	...	...	...	1	...	...	...	...	...	2	...	...	5	...
7·0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	...	...	...	4	...	...	7	1
6·8	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	...	...	...	1	3	1	1	1	1	...	1	1	1	...	...	...	...	...	...	...	...	...	...	1	...	...	...	...	...	1	...	...	15	...
6·6	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	1	1	1	1	1	1	...	...	...	1	3	1	...	...	1	...	...	...	2	...	...	...	...	...	1	...	...	13	2
6·4	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	...	...	1	...	3	...	1	...	3	3	...	...	2	1	1	...	...	1	...	1	...	1	...	2	...	...	...	...	...	2	...	...	21	4
6·2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	2	2	2	1	...	1	2	...	2	4	2	2	2	2	...	...	...	...	...	...	...	...	...	...	5	...	...	27	3
6·0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	2	2	...	...	1	...	1	1	...	3	1	1	1	1	2	1	...	1	1	1	...	...	1	1	1	...	...	...	...	...	...	...	...	20	3
5·8	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	1	1	...	...	1	1	2	...	2	3	2	4	4	1	1	1	4	...	1	...	3	1	...	...	...	...	...	...	...	...	...	...	2	...	...	24	12
5·6	...	...	...	...	...	...	...	...	...	...	...	...	...	2	...	1	...	...	1	...	...	1	2	...	1	...	1	...	3	2	1	...	2	2	1	...	3	...	...	...	...	...	...	...	...	...	...	...	...	1	1	...	...	10	1
5·4	...	...	...	...	...	...	...	...	...	...	...	...	1	...	1	1	...	1	4	...	5	2	...	...	3	2	2	2	3	1	...	1	...	...	...	2	...	...	1	...	...	...	...	...	1	...	...	...	...	...	...	...	25	8
5·2	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	1	1	...	2	2	1	1	1	3	1	1	1	...	3	1	...	...	1	1	...	...	...	1	...	...	...	...	...	...	...	...	1	...	...	1	...	...	22	2
5·0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	1	1	1	3	2	...	1	...	...	1	1	...	...	...	...	2	...	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	...	11	4
4·8	...	...	...	...	...	...	...	...	1	...	1*	...	...	...	...	2	2	...	1	2	...	...	...	...	1	...	1	...	...	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	1	8	2
4·6	...	...	...	...	...	...	...	1	...	...	...	...	...	...	1*	...	1	1	...	...	...	...	...	...	...	...	...	2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	2	1	2	1
4·4	...	...	...	...	...	...	1	...	2*	1	...	...	1*	...	1	1*	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	7	1	...	...
4·2	...	...	...	...	...	...	...	2	1	...	...	2	5*	3	...	1	...	...	...	...	2	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	11	3	2	1
4·0	...	...	...	...	...	1	...	3	5	...	2	4	1	1	...	2	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	15	5	...	...
3·8	...	...	...	...	2	1	4	5	2	1	3	4	2	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	16	9	...	...
3·6	1	...	...	2	...	1	...	...	2	...	...	1	1	...	...	1	...	1	...	...	...	...	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	4	6	...	1
3·4	...	...	3	...	1	1	...	...	3	...	...	1	1	...	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	4	7	...	...
3·2	...	...	1	2	...	...	2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	4	...	...
$L^N K$	...	...	2	1	1	2	5	8	9	1	2	7	9	2	2	5	3	2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	61	...	...	...
$L^N k$	1	...	2	3	2	2	2	3	7	1	4	5	2	2	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	37	...	...
$L^L K$	...	...	...	...	...	...	...	...	...	...	...	...	...	3	1	1	3	6	6	7	10	3	7	4	9	10	11	8	14	14	12	6	5	9	14	9	9	5	10	3	5	1	4	6	...	1	5	...	1	25	...	...	247	...
$L^L k$	...	...	...	...	...	...	...	...	...	...	...	...	1	...	1	2	...	...	3	...	4	4	...	2	2	...	2	5	3	2	2	1	1	4	3	3	2	1	...	1	...	1	...	...	1	...	1	...	...	3	...	...	...	55

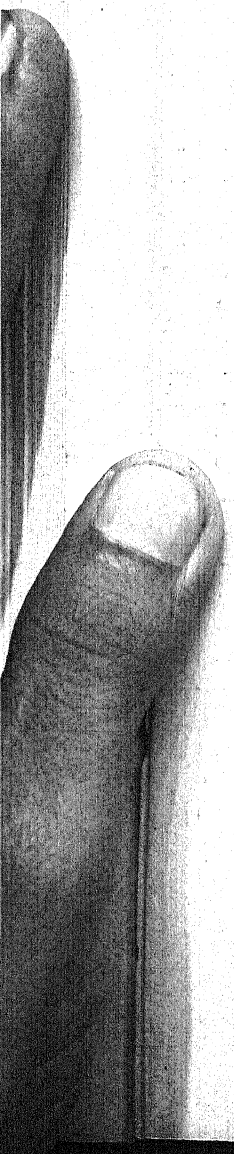

TABLE VI

Correlation between index A and index B in (1090 × C. W.) C. W.

		Index A																Totals	
		2·4	2·6	2·8	3·0	3·2	3·4	3·6	3·8	4·0	4·2	4·4	4·6	4·8	5·0	5·2	5·4	5·6	+	0
Index B	4·2	...	...	...	...	...	1	...	...	...	...	...	...	...	...	1	1	...	1	2
	4·0	...	...	...	...	...	4	...	...	...	...	...	...	1	...	...	1	1	4	3
	3·8	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	1	...	1	1
	3·6	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...
	3·4	...	...	...	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	1
	3·2	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...
	3·0	...	...	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	...	1
	2·8	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
	2·6	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
	2·4	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1
Totals	+	...	...	2	...	...	6	...	...	...	...	...	...	...	...	...	...	...	8	...
	0	1			...	...	...	...	1	1	...	...	...	1	...	1	3	1	...	9

TABLE VII

Correlation between index A and index B in (1090 × Kokati narrow) M9

Index B	Index A 2·0	2·2	2·4	2·6	2·8	3·0	3·2	3·4	3·6	3·8	4·0	4·2	4·4	4·6	4·8	Totals +	?	0
5·0	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	1	...	...
4·8	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
4·6	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
4·4	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	...	1	...
4·2	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	1	...	...
4·0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
3·8	...	...	...	...	...	...	1	...	...	...	...	...	1	1	1	1	...	3
3·6	...	...	1	2	3	1	2	...	1	1	...	3	...	1	3	7	5	6
3·4	...	...	1	3	1	1	...	...	...	2	3	3	...	1	...	6	1	8
3·2	...	3	2	...	2	...	...	...	...	3	...	...	...	...	1	6	3	2
3·0	...	...	1	...	...	1	...	...	...	...	1	...	...	...	...	2	1	...
2·8	...	...	3	...	1	...	...	...	...	...	...	...	...	...	...	3	...	1
2·6	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...
2·4	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	1	...	...
+	1	3	8	7	4	4	2	...	...	...	...	...	...	...	...	29	...	...
?	...	...	...	...	2	...	1	...	...	2	2	1	...	...	3	...	11	...
Totals 0	...	...	...	...	1	...	...	...	1	4	2	6	1	3	2	...	...	20
Brown	1	2	6	3	1	3	2	...	...	2	1	...	...	...	1	22	Brown	
White	...	1	2	4	6	1	1	...	1	4	3	7	1	3	4	38	White	

TABLE VIII

Correlation between index A and index B in (1090 × 1304) C. W.

Index B	Index A: 2·8	3·0	3·2	3·4	3·6	3·8	4·0	4·2	4·4	4·6	4·8	5·0	5·2	5·4	5·6	5·8	6·0	6·2	6·4	6·6	Totals +	Totals 0
4·2	...	...	...	...	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	1
4·0	...	...	...	...	...	...	...	...	...	...	1	...	1	...	1	1	...	1	1	1	...	7
3·8	...	...	...	1	...	...	...	...	...	...	...	...	...	1	1	1	...	...	...	...	1	3
3·6	...	...	...	...	...	...	1	...	...	...	...	1	2	2	4	...	1	...	...	...	1	10
3·4	1	...	1	...	1	...	...	...	...	...	...	...	1	...	...	...	...	...	...	...	3	1
3·2	...	1	1	1	3	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	7	...
3·0	...	2	1	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	2	2
2·8	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...
Totals +	1	2	4	2	4	...	2	...	...	...	...	...	4	3	6	2	1	1	1	1	15	...
Totals 0	...	1	...	...	...	...	1	...	...	1	1	1	...	...	...	...	...	...	...	...	...	24

TABLE IX

Correlation between index A and index B in (1090 × Kokati broad) M9

		Index A																Totals		
		1·8	2·0	2·2	2·4	2·6	2·8	3·0	3·2	3·4	3·6	3·8	4·0	4·2	4·4	4·6	4·8	+	?	0
Index B	4·4	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	1	...	...	2
	4·2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
	4·0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	1
	3·8	...	...	...	...	...	...	...	...	...	...	...	2	...	1	1	...	...	1	3
	3·6	...	...	...	...	...	...	...	...	...	...	1	1	2	2	...	...	...	1	5
	3·4	...	...	...	...	1	...	...	...	...	...	...	1	1	2	3	...	1	1	6
	3·2	...	...	1	1	1	...	...	...	...	...	1	1	...	...	...	...	3	...	2
	3·0	...	1	...	...	...	...	1	...	...	1	...	...	...	...	...	...	1	...	2
	2·8	2	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	2	...	1
	2·6	...	2	...	3	...	...	...	...	...	...	...	...	...	...	...	...	4	...	1
Totals	+	1	3	1	4	2	...	...	...	...	...	...	...	...	...	...	...	11	...	...
	?	...	...	...	...	...	...	...	...	...	...	...	1	1	1	...	...	...	3	...
	0	1	1	...	...	...	...	1	...	...	1	2	4	2	4	5	2	...	...	23

THE GENETICS OF A PETALOID MUTANT IN COTTON

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(Received for publication on 7th April 1938)

(With Plate XXXVIII)

INTRODUCTORY

MUTATIONS in cottons are not of rare occurrence. Several mutations have already been described by various workers, and the work on the genetics of such mutations has added a great deal to our knowledge of heredity of this very important plant. One mutation occurred at the Cotton Research Station, Lyallpur, and a record of its genetic behaviour is discussed in the present article.

The mutation in question was the occurrence of petaloid flowers in a pure strain of *G. arboreum, forma bengalensis* var. *sanguineum, neglecta* H & G. This pure strain had been grown at the Cotton Research Station, Lyallpur, for ten successive years from self-bred seed and had perfectly normal flowers. The mutation under discussion occurred, probably, during the year 1933, but was not detected until 1935. During this year the self-bred seed of one plant (06/34 P 12) was grown at Lyallpur and the natural seed of the same was grown simultaneously at the Cotton Research Sub-Station, Multan. The progeny of this plant at both the places exhibited three distinct types of plants, namely, with 'complete' petaloid flowers, intermediate flowers and normal flowers in a ratio approximately to 1 : 2 : 1. Since the number of the plants during this year was only 14 at Lyallpur, not much reliance could be placed on this ratio. The counting of plants of different types at Multan was, however, not done.

DESCRIPTION

The intermediate and the 'complete' petaloid plants are absolutely indistinguishable from the normal plants until the flowers open. In 'complete' petaloid plants almost all the stamens are transformed into structures resembling petals. Both the filaments and anthers are dilated to form such structures. This type of metamorphosis occurs right up to the top of the staminal column, leaving just a few normal stamens at the top. These few

anthers produce perfectly viable pollen. The ovules in all cases are normal and it is possible to make reciprocal crosses between the normals, 'complete' petaloid and the 'intermediate' plants.

In the intermediate plants the anthers are transformed into petal-like structures only on the lower half of the staminal column. The anthers on the upper half are normal and functional.

The plants with normal flowers are like ordinary plants in all respects.

The three types of flowers are represented in Plate XXXVIII.

Literature

Saunders [1910-11] in Petunia, Gregory [1910] in *Primula sinensis*, Miyake and Imai [1928] in the Japanese Morning Glory and Ramanathan and Sankaran [1934] in cotton have reported double flowers being recessive to normal. Hutchinson and Ghose [1937] have, however, reported that the dominance of normal in the case of cotton is incomplete. The segregation in the F_2 generation of the double flower in *Primula sinensis*, Japanese Morning Glory and cotton has been reported to be in a monohybrid ratio, but in Stock a dihybrid ratio has been recorded.

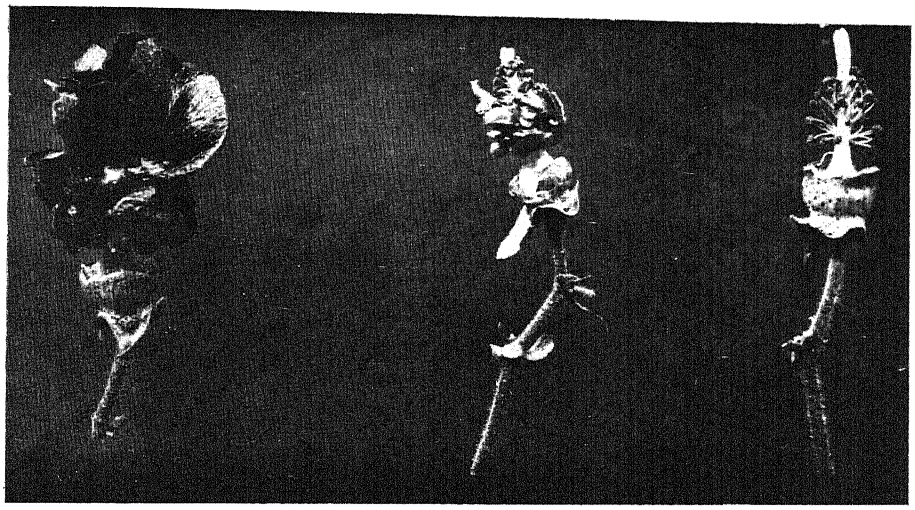
Experimental results

From the mixed population of the 1935 crop, enough seed was selfed on the plants of all the three types, namely, normals, intermediates and 'complete' petaloids, in order to grow large progenies in the following year. Reciprocal crosses of normal × intermediate, intermediate × 'complete' and normal × 'complete' were also made. The self-bred seed obtained from the three types of plants and their reciprocal crosses was grown during 1936 and of their derivatives in 1937 and 1938. The analysis of the progenies from the Self-bred seed in the various years is given in Table I.

Table I

Classification of progenies of normal, intermediate and 'complete' petaloid plants

Year	Parent plant	No. of families grown	Total No. of population	Classification			Value of P of χ^2
				Normal	Intermediate petalody	Complete petalody	
1936	Normal	2	47	47	...	...	...
1937	Do.	1	18	18	...	...	...
1938	Do.	1	24	24	...	...	...
1936	Complete petalody	2	38	...	...	38	...
1937	Do.	2	67	...	...	67	...
1938	Do.	1	24	...	...	24	...
1936	Intermediate petalody	3	50	11	24	15	1:2:1=0·70
1938	Do.	6	399	106	203	90	1:2:1=0·7



Complete Intermediate Normal

Three types of flowers after removing the calyx and the corolla

It will be seen from Table I that the intermediate petaloid plants segregated in 1 : 2 : 1 ratio, while the ' complete ' petaloid and the normal plants bred true. Further corroborative evidence for monohybrid segregation was forthcoming from the crosses the data for which are shown in Table II.

TABLE II

Segregation in the progenies of the crosses

Year	Cross	No. of families grown	Total No. of population	Classification			Value of P of χ^2
				Normal	Intermediate petalody	Complete petalody	
1936	Normal × intermediate petalody and reciprocal	4	230	126	104	...	1 : 1 = 0·15
1936	Intermediate petalody × complete petalody and reciprocal	2	114	...	64	50	1 : 1 = 0·2
1936	Normal × complete petalody and reciprocal	4	202	...	202	...	...
1937	Ditto	4	606	...	606	...	...
1938	(Complete petalody × normal) × normal	2	128	67	61	...	1 : 1 = 0·6

The evidence presented above shows that a single-factor difference was involved, but the dominance of the mutant character (complete petalody) over the normal was incomplete.

DISCUSSION

The results presented above show definitely that the mutant petaloid plants differed from the normals by a single factor. The origin of this mutation is not very clear. Since self-bred seed had been used for ten successive generations prior to the occurrence of the mutation, it can be assumed that the material was homozygous in most characters. It can, perhaps, be assumed that the parent plant of 1934 became heterozygous by mutation, and this escaped notice during 1934. In 1935 the segregation in a 1 : 2 : 1 ratio was detected.

The investigation reported here is of special interest in so far as the mutant petaloid condition is incompletely dominant over the normal flower. The investigators on the other plants have found such mutations recessive to normal. The dominance of ' normal ' in the case of cotton has elsewhere been proved to be incomplete [Hutchinson and Ghose, 1937, 1]. The mutant plants, both male and female, are fertile and are able to set seeds.

Another important phenomenon noticed during this experiment was the weakening of the petaloid character with the advance of the season. In the beginning of the season when the plants were in the phase of active growth, the flowers were completely petaloid. As the season advanced, this character weakened and by the end of October the complete petaloid plants could be distinguished from the intermediate ones of a month ago with great difficulty.

This weakening of the petaloid character with the change in the season has already been reported by Miyake and Imai [1928] in Morning Glory. In cotton Hutchinson and Ghose [1937, 2] have also reported a similar phenomenon when the 'crinkled' *hirsutum* plants were grown inside the greenhouse. Thus the impact of the environmental conditions appears to have a far-reaching effect on these mutations. The explanation of this phenomenon can be found in the main mutating gene being attended by some modifying genes which find expression only in a limited set of conditions. This point is important and is worth noting in classifications of progenies for genetic analysis. If the analysis is made rather late in the season the distinction between full petaloid and intermediate is likely to be lost sight of and lead to misleading ratios of the different types. A similar case has already been reported by one of us [Afzal, 1937] where serious discrepancies were liable to occur in the ratios due to the classifications of the progenies being done at the wrong time.

Further work on this problem is in progress and the Madras petaloid in Karunganni cotton has been crossed with the Lyallpur petaloid. The data of this cross are so far incomplete and will be reported later.

Summary

The mutation of double flower in cotton discussed in this paper is of incompletely dominant nature.

Both male and female gametes are normally functional even in the complete petaloid plants.

Petalody in cotton constitutes a single-factor difference to normal and the inheritance is in the ratio of 1 : 2 : 1.

Petalody becomes weak with the advance of the season, due, probably, to the interaction of modifying genes, which are effective only under a certain set of environmental conditions.

Acknowledgments

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VARIATION IN *ASCOCHYTA RABIEI* (PASS) LAB., THE CAUSAL FUNGUS OF BLIGHT OF GRAM (*CICER ARIETINUM* L.)

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(With three text-figures)

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I. Introduction

THE investigations of the blight disease of gram (*Cicer arietinum* L.) caused by *Ascochyta rabiei* Pass (Lab.) which is very serious in the Punjab were started in 1926. The work done on the study of the life-history, modes of perpetuation of the causal fungus and measures of control of the disease has been already published [Luthra and Bedi, 1932; Sattar, 1932, 1933; Luthra, Sattar and Bedi, 1935]. The measures recommended to control the disease consisted in sowing healthy seed and destruction of diseased plant debris from the fields. But in order to check effectively the disease these measures have to be carried out thoroughly in cooperation by all farmers

in an affected locality. In practice, however, it has been found difficult to get them to adopt these methods. Under favourable conditions the disease has often assumed epidemic form and become widespread over gram-growing areas. Under these circumstances, therefore, the final solution of the problem of blight control was considered to be in the production of types of gram resistant to blight. In this connexion it was necessary, first of all, to find out if the gram blight fungus in the Punjab consisted of any biological strains. Work was, therefore, undertaken to study the strains of *Ascochyta rabiei*.

II. Experimental

1. Material and methods

About 2,000 isolations were made from diseased material of gram, consisting of branches, pods, seeds and leaflets collected from different places—Campbellpur, Rawalpindi, Jhelum and Lyallpur. No indication was obtained that certain forms were exclusively confined to particular organs of the gram plant or to a particular locality. In all, six forms appeared to be distinct and these were included in the study. These are designated as A, B, C, D, E and F.

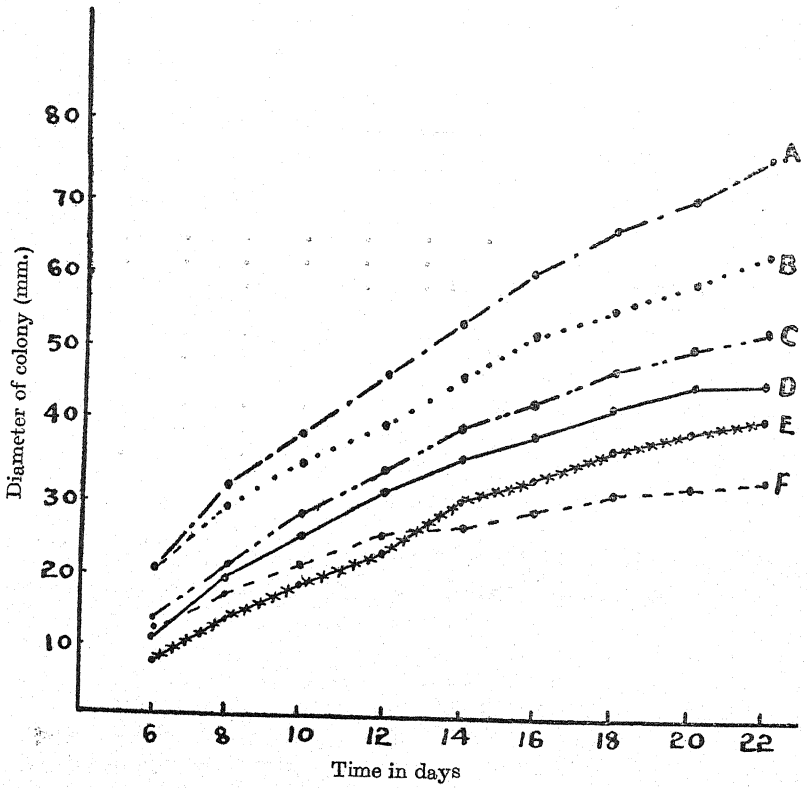

Fig. 1. Colony growth of the six forms of *A. rabiei* on Richards' agar at 20°C.

The following artificial culture media were used for growing the different forms. The nutrients were made up in one litre solution in distilled water :—

Oatmeal agar	Oatmeal 50 gm., and agar agar 20 gm.
Richards' agar	Sucrose 50 gm., KNO_3 10 gm., KH_2PO_4 5 gm., $MgSO_4$ 2·5 gm. and agar agar 20 gm.
Nutrient glucose agar	Glucose 20 gm., peptone 10 gm., NaCl 5 gm., extract of meat 4 gm. and agar agar 15 gm.
Dolt's agar	Glycerine 5 gm., ammonium phosphate 1 gm., lactose 10 gm., and agar agar 15 gm.
Brown's agar	Glucose 2 gm., asparagin 2 gm., K_2PO_4 1·25 gm., $MgSO_4$ 0·75 gm., and agar agar 15 gm.

2. Study of the six forms in artificial culture media

(*A*) *Microscopic growth features*

(*i*) Linear growth rate : *Effect of culture media.*—The six forms were grown on the five culture media at 20°C. and the diameter of colony of each form was recorded on every alternate day. The relative growth rate of the six forms on Richards' agar is shown in Fig.1 from which it will be seen that as regards diameter of the colony the different forms fall in the order A, B, C, D, E and F where A has the greatest linear growth and F the least.

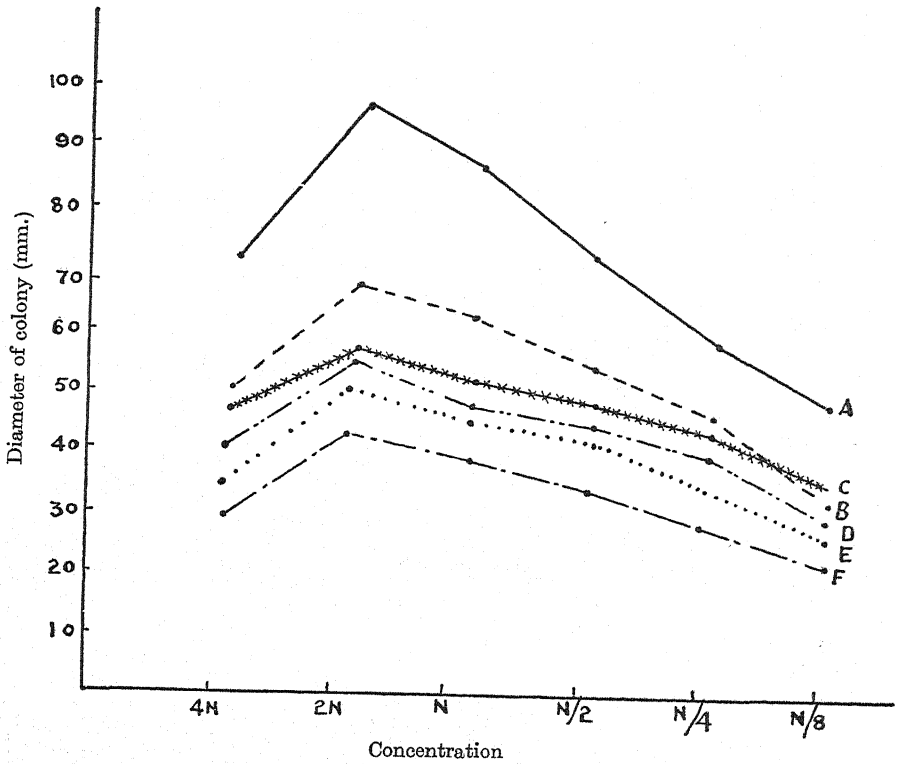

Fig. 2. Effect of concentration of culture medium (nutrient glucose agar) on colony growth of the six forms of *A. rabiei* at the end of three weeks at 20°C.

Similar curves were obtained for the linear growth of these forms on oatmeal agar, nutrient glucose agar, Dolt's agar and Brown's agar, showing thereby that all the forms are fairly distinct with regard to their growth rates.

Effect of concentration of the medium :—In order to test the effect of concentration of the medium, nutrient glucose agar was used. A range of concentrations from 4 N* to $N/8$ was prepared and the six forms were grown at 20°C. The diameter measurements of colonies of each form at the end of three weeks on the various concentrations of the medium are shown in Fig. 2.

From Fig. 2 it is clear that all the forms have their maximum growth at 2 N and their growth above this point falls. At all the concentrations their order with regard to growth rate is the same as on different culture media referred to above except a slight variation at $N/8$ where B and C have changed places.

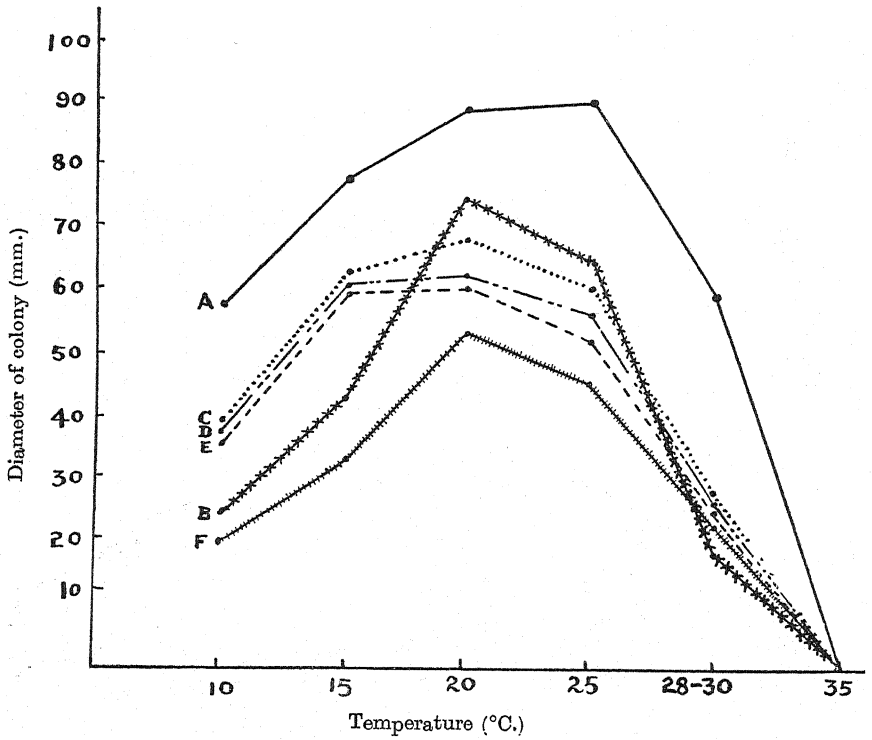

FIG. 3. Effect of temperature on colony growth of the six forms of *A. rabiei* on oatmeal agar at the end of three weeks

* N means normal nutrient glucose agar, $2N$ means double the quantity of the nutrients in 1 litre solution and $N/2$ means half the quantities of the nutrients in 1 litre solution and so on.

Effect of temperature.—The various forms were grown on oatmeal agar at 10°, 15°, 20°, 25°, 28°-30° and 35°C. for three weeks and the final colony diameter of each form is shown in Fig. 3.

From the results it is clear that the optimum temperature for growth of forms B, C, D, E and F is about 20°C. while that of A is about 25°C. and the maximum for all the forms is between 30° and 35°C.

Here again at all the temperatures the various forms excepting B arrange themselves in the same order with regard to growth rate as they do on the various media and on different concentrations of nutrient glucose agar referred to above. Form B maintains that order at 20°C. and 25°C., but at temperatures higher and lower than this range its growth falls more markedly than the other forms.

(*ii*) SPORULATION (PYCNIDIAL FORMATION): *Effect of culture medium.*—The relative amount of pycnidia formation of the six forms after three weeks' growth on different culture media is given in Table I from which it is clear that forms A and D are characterized by profuse sporulation, forms C and E are moderately fertile, while forms B and F are rather poor spore producers.

TABLE I

Relative amount of sporulation of the six forms on four culture media

Form	Richards' agar	Nutrient glucose agar	Dolt's agar	Brown's agar
A	++++	++++	+++	++
B	+	+	+	+
C	+++	+++	++	+
D	++++	++++++	++++	+++
E	++	+++	++	++
F	—	+	+	+

— = Absent, + = Scanty, ++ = Moderate, +++ = Good, ++++ = Very good, ++++++ = Abundant.

Effect of concentration of medium:—The six forms were grown on different concentrations of nutrient glucose agar at 20°C. and the relative amount of pycnidial formation was recorded after three weeks. The results are given in Table II.

From the results given in Table II it will be seen that at *N* the sporulation of all the forms is the maximum, above this point sporulation decreases very rapidly so that at 2*N* forms B and F do not produce any pycnidia and at 4*N* all the forms excepting D remain sterile. Below *N* the amount of sporula-

tion decreases but not rapidly. It is noteworthy that at different concentrations of nutrient glucose agar just as on different media the forms B and F sporulate less abundantly than the other forms.

TABLE II

Relative amount of sporulation of the six forms on different concentrations of nutrient glucose agar

Form	4*N*	2*N*	*N*	*N*/2	*N*/4	*N*/8
A	—	+	++++++	++++	+++	++
B	—	—	++	++	++	+
C	—	+	++++++	++++	+++	++
D	+	+++	+++++++++	++++++	++++	+++
E	—	+	++++	+++	++	+
F	—	—	Traces	+	++	+

— = Absent, + = Scanty, ++ = Moderate, +++ = Good, ++++ = Very good, ++++++ = Abundant, +++++++++ = Very abundant.

Effect of temperature:—The effect of temperature on sporulation is recorded in Table III from which it will be seen that the temperatures from 10°C. to 25°C. are in general favourable for pycnidial formation for all the forms. At 30°C. form A sporulates profusely, forms B, C and F remain sterile, while forms D and E produce scanty pycnidia. Here again it is clear that forms B and F are rather poor spore formers.

TABLE III

Relative amount of sporulation of the six forms on nutrient glucose agar at different temperatures

Form	10°C.	15°C.	20°C.	25°C.	29°C.	35°C.
A	++++++	+++++++++	+++++++++	+++++++++	++++	No growth
B	+	+	++++	+++	—	
C	+++	++++++	+++++++++	++++++	—	
D	+++++++++	++++++++++	++++++++++	++++++++++	+	
E	++++	++++++	+++++++++	++++++	+	
F	—	+	++	+	—	

— = Absent, + = Scanty, ++ = Moderate, +++ = Good, ++++ = Very good, ++++++ = Abundant, +++++++++ = Very abundant, ++++++++++ = Super-abundant.

(*iii*) AERIAL MYCELIUM: *Effect of culture medium.*—The relative amount of aerial mycelium of the six forms on the four culture media at 20°C. at the end of three weeks is given in Table IV. It will be seen from this table that of all the forms, form B produces thick, profuse aerial mycelium on all the media tried, while the other forms are characterized by meagre or no development of aerial mycelium.

TABLE IV

Relative amount of aerial mycelium of the six forms on different culture media

Form	Richards' agar	Nutrient glucose agar	Dolt's agar	Brown's agar
A	—	Abundant to start with, but autolysed eventually.	Abundant to start with, but autolysed eventually.	
B	+++	++++	++	++
C	+	—	—	—
D	—	—	—	—
E	—	—	+	—
. . . . .	+	+	+	+

— = Absent, + = Scanty, ++ = Moderate, +++ = Good, ++++ = Very good.

Effect of concentration of a medium.—The relative amount of aerial mycelium of the six forms on different concentrations of nutrient glucose agar at 20°C. after three weeks' growth is given in Table V. Though at *N* and lower concentrations forms B and F only produce aerial mycelium, yet at 2*N* and 4*N*, the other forms also develop this character.

TABLE V

Relative amount of aerial mycelium of the six forms on different concentrations of nutrient glucose agar

Form	4*N*	2*N*	*N*	*N*/2	*N*/4	*N*/8
A	++++	+++	—	—	—	—
B	+++++++	++++++	++++	++	+	+
C	++++	+	—	—	—	—
D	+	+	—	—	—	—
E	++	+	—	—	—	—
F	++++	+++	++	+	—	—

— = Absent, + = Scanty, ++ = Moderate, +++ = Good, ++++ = Very good, ++++++ = Abundant, +++++++ = Very abundant.

Effect of temperature.—The effect of temperatures varying from 10 to 30°C. was studied on the production of aerial mycelium by the six forms. At all the temperatures form B produces profuse aerial mycelium, form F scanty, while the other forms do not develop any aerial mycelium.

(*iv*) COLONY COLOUR.—The colony colour of the various forms after three weeks' growth on different media at 20°C. at the end of three weeks is given in Table VI.

TABLE VI

Colony colour of the six forms on different culture media

Form	Richards' agar	Nutrient glucose agar	Dolt's agar	Brown's agar
A	Deep mouse grey to dark mouse grey	Almost black	Pale pinkish buff before autoloysis, pale flesh after autoloysis	Pale mouse grey to light mouse grey, with a pinkish hue
B	Mouse grey to smoke grey	Greyish olive	Deep olive buff	Smoke grey
C	Black	Black	Black	Black
D	Light pinkish cinnamon	Pinkish cinnamon	Pinkish buff	Pinkish buff
E	Pallid mouse grey to deep mouse grey	Black	Olivaceous black	Black
F	Vinaceous pink	Pinkish buff	Pinkish buff	Vinaceous pink

It will be seen that though the culture medium exerts a noticeable influence on the colony colour, yet the change is of degree only and the various forms remain fundamentally unaltered on the different media, except form A which on three culture media, viz. Richards' agar, nutrient glucose agar and Brown's agar, produces almost the same colour, but on Dolt's agar exhibits different colour altogether.

(*B*) *Microscopic growth features*

Cultures of the six forms grown on oatmeal agar for three weeks at 20°C. were used for microscopic studies given below.

One hundred pycnidia and 100 pycnospores of each form were measured at random.

(*i*) PYCNIDIA.—Pycnidia of the different forms are generally round when single, but of irregular shape when in aggregation. The peridium consists of polygonal pseudo-parenchymatous cells. The ostiole may be present or absent in cultures. The pycnidia of forms A, B, C, E and F are deep olive in colour but those of D are pinkish buff. The size of 100 pycnidia of each of the six forms is given in Table VII.

TABLE VII

Size of pycnidia in microns of the six forms on oatmeal agar at 20°C.

Form	Range in microns	Mean in microns
A	30—240 × 30—210	116 × 100
B	105—330 × 105—270	207 × 188
C	75—330 × 60—300	181 × 160
D	75—480 × 75—480	250 × 223
E	90—375 × 75—300	219 × 197
F	45—285 × 45—225	132 × 119

The data on the size of pycnidia were examined statistically and the analyses of variance are given in Tables VII (a) and VII (b).

TABLE VII (*a*)

Analysis of variance of length of pycnidia

Due to	Degrees of freedom	Sums of squares	Mean square
Blocks	9	7325·95	813·99
Treatments	5	134154·02	26830·80
Error	45	38350·78	852·23
Total	59	179830·75	

TABLE VII (*b*)

Analysis of variance of breadth of pycnidia

Due to	Degrees of freedom	Sums of squares	Mean square
Blocks	9	6010·30	667·81
Treatments	5	111707·30	22341·46
Error	45	36293·50	806·52
Total	59	154011·10	

From Fisher's z-table it is clear that if the results are to be significant the mean square due to treatments should be at least 3·45 times the mean square due to error. Hence the results are highly significant in both cases. Further analysis of each form was made and the results are that as regards length of pycnidia, the differences between A and B, A and C, A and D, A and E, B and C, B and D, B and F, C and D, C and E, C and F, D and E, and E and F are significant, but the differences between A and F, B and E, and D and E are not significant. As regards breadth of pycnidia, the differences between A and B, A and C, A and D, A and E, B and D, B and F, C and D, C and E, C and F, D and F, and E and F are significant, while the differences between A and F, B and C, B and E, and D and E are not significant. Thus with regard to length of pycnidia, the forms can be grouped as follows :—

(*i*) A and F

(*ii*) C

(*iii*) B, D and E

With regard to breadth of pycnidia they fall into two groups :—

(*i*) A and F

(*ii*) B, C, D and E

(*ii*) PYCNOSPORES.—The pycnospores of all the forms are hyaline, generally single-celled, oval to oblong with muticle ends. About one per cent. pycnospores with a faint medium septum are observed in culture.

Size.—The size of 100 pycnospores of each of the six forms is given in Table VIII.

TABLE VIII

Size of 100 pycnospores of six forms on oatmeal agar at 20°C.

Form	Length in microns		Width in microns		Length / Width
	Range	Mean	Range	Mean	
A	5-10	8·1	3·5-5·0	4·0	2
B	4-17	11·78	2·5-6·5	4·68	2·5
C	4-16	10·01	3·0-6·5	3·95	2·5
D	4-24	11·56	2·5-6·5	4·0	2·8
E	4-16	11·28	2·0-6·0	4·29	2·6
F	3-18	12·68	3·0-10·0	5·23	2·4

The data on the size of pycnospores were examined statistically and the analyses of variance are given in Tables VIII (*a*) and VIII (*b*).

TABLE VIII (*a*)

Analysis of variance of the length of pycnospores

Due to	Degrees of freedom	Sums of squares	Mean square
Blocks	9	4·95	0·55
Treatments	5	121·29	24·25
Error	45	40·94	0·909
Total	59	167·18	

TABLE VIII (*b*)

Analysis of variance of the breadth of pycnospores

Due to	Degrees of freedom	Sums of squares	Mean square
Blocks	9	0·65	0·07
Treatments	5	10·38	2·07
Error	45	3·72	0·082
Total	59	14·75	

From Fisher's *z*-table it is clear that if the results are to be significant the mean square due to treatments should be at least 3·45 times the mean square due to error. Hence the results are highly significant in both the cases.

Further analysis of each form was made and the results are that as regards length of pycnospores, the differences between A and B, A and C, A and D, A and E, A and F, B and C, C and D, C and E, C and F, and E and F are significant, whereas differences between B and D, B and E, B and F, D and E, and D and F are not significant. As regards breadth of pycnospores the differences between A and F, B and C, C and F, D and F, and E and F, are significant, but differences between A and B, A and C, A and D, A and E

B and D, B and E, B and F, C and D, C and E, and D and E are not significant. Thus with regard to length of pycnospores the forms can be grouped as follows :—

(*i*) A

(*ii*) C

(*iii*) B, D, E and F.

With regard to breadth all the forms are identical.

Shape.—The ratio length/width of the pycnospores of the six forms is given in the last column of Table VIII. This ratio is the smallest in form A. Therefore its pycnospores tend to be oval. Length/width in form D is the largest, therefore its pycnospores are somewhat rod-like and slender. Form F has the longest pycnospores which also have the greatest width and, therefore they tend to be more oval than oblong. Form C has its pycnospores intermediate both in length and breadth and thus most of its pycnospores are oval to oblong.

Germination.—Germination of pycnospores of all the six forms is negligible (only 1-2 per cent) in distilled water. They germinate well in *N*/25 to *N*/100 malic acid and thus the results already recorded by Sattar [1933] are confirmed. Gram leaf extract, which in addition to malic acid also contains some other nutrients, is better than malic acid for their germination but as the gram leaves are not available throughout the year and composition of gram leaf extract is variable, *N*/50 malic acid was used for germination tests. Data on the percentage of germination and length of germ-tubes are recorded in Table IX.

TABLE IX

Germination studies of the six forms in N/*50 malic acid at 20°C. at the end of 16 hours*

Form	Percentage of germination	Percentage of bicellular spores	Average germ-tube length in microns
A	100·0	0·4	128·95
B	100·0	8·0	57·0
C	97·6	2·0	59·6
D	100·0	7·4	59·46
E	98·4	9·6	55·2
F	100·0	11·8	47·82

With regard to the mode of germination all the strains are almost identical. Germination was found to have commenced after four hours in nearly all the forms. By the end of eight hours a large percentage of spores had germinated.

It will be seen from Table IX that the length of germ-tubes of spores of form A is almost double that of spores of the other forms, which are almost identical in this respect. During germination about 2-12 per cent spores of forms B, C, D, E and F become bicellular but only 0·5 per cent pycnospores of form A develop a median septum.

(*iii*) MYCELIUM.—With regard to colour of mycelium under the microscope the various forms fall into two distinct groups.

(1) Mycelium almost hyaline or colourless.

(2) Mycelium highly pigmented.

To group (1) belongs form D which has delicate and almost colourless and sparsely septate mycelium. To group (2) belong all the remaining forms. In the case of black forms A, C and E nearly the entire fungal mat consists of highly pigmented (deep olive to dark olive), closely septate and sparsely branching mycelium. In the case of forms B and F the aerial mycelium remains hyaline for some time but becomes deep olive in the end. However the compact fungal mat underlying the aerial mycelium is always dark.

3. PATHOGENICITY

For testing the pathogenicity of the various forms, potted gram (*Cicer arietinum*) plants of Punjab types 1-26 were inoculated by spraying spore suspension of the various forms. After inoculation the plants were kept covered for four days with disinfected glass bell-jars. All the forms except A produced infection on all the types of gram inoculated. Each form that caused infection was re-isolated from the diseased plants and it was observed that each form after having passed through the gram plant maintained its individuality. The symptoms and the amount of infection caused by the five forms, B, C, D, E and F on all the types of gram inoculated were similar. It therefore appears that the five forms B, C, D, E and F are biologically indistinct.

III. Discussion

The cultural as well as the morphological characters of the six forms A, B, C, D, E and F which have been studied are summarized in Table X.

From Table X it is evident that form A differs markedly from all the other forms, excepting F, in respect of size of pycnidia and from all the other forms in respect of length of pycnospores, germ-tube length, linear growth rate, response to temperature and its non-pathogenicity on gram plants. This form, therefore, does not belong to the species *Ascochyta rabiei*. It only occurs as a saprophyte on gram.

TABLE X

Summary of the cultural and morphological characters of the six forms

Character \ Form	A	B	C	D	E	F
Linear growth on Richards' agar after 22 days in mm.	75	62	52	46	40	33
Optimum temperature for growth (°C.)	25	20	20	20	20	20
Sporulation . . .	Profuse	Poor	Moderate	Profuse	Moderate	Poor
Aerial mycelium . . .	Poor	Profuse	Meagre	Nil.	Meagre	Meagre
Colony colour . . .	Deep mouse grey	Mouse grey	Black	Pink	Mouse grey	Pink
Size of pycnidia in microns .	116×100	207×188	181×160	250×223	219×197	132×119
Size of pycnospores in microns.	8·1×4·0	11·7×4·6	10·0×3·9	11·5×4·0	11·2×4·2	12·6×5·2
Septation of pycnospores before germination.	100 per cent unicellular	99 per cent unicellular and 1 per cent bicellular	99 per cent unicellular, 1 per cent bicellular	99 per cent unicellular. 1 per cent bicellular	99 per cent unicellular, 1 per cent bicellular	99 per cent unicellular, 1 per cent bicellular
Percentage of bicellular pycnospores after germination.	0·4	8·0	2·0	7·4	9·6	11·8
Germ-tube length in microns .	128·9	57·0	59·0	59·4	55·2	47·8
Pathogenicity on Punjab type (26) of gram.	Negative on all	Positive on all	Positive on all	Positive on all	Positive on all	Positive on all

The other five forms belong to the species *Ascochyta rabiei*. These are parasitic on the gram plant and produce the same symptoms. On morphological basis (length of pycnospores and pycnidia) the form C can be distinguished from the other forms. The form C can also be distinguished from the other forms by the black colour of its colony. But the other four forms cannot be differentiated from one another. All the five forms are biologically identical. The authors, therefore, do not suggest that form C should be regarded a separate variety of *A. rabiei*.

IV. Summary

1. Six forms were isolated from diseased gram plants affected with *Ascochyta rabiei*. Detailed studies showed that five of these forms distinguished as B, C, D, E and F belonged to *Ascochyta rabiei* and one form designated as A occurred on gram plants as a saprophyte.

2. A comparative study of all the six forms has been made as regards the following :—

(*a*) Morphology on the various artificial culture media,

(*b*) Response to environmental conditions, such as nature of medium and temperature,

and

(*c*) Manner of spore germination.

3. Inoculation experiments with all the six forms have been done on potted gram plants and the result obtained is that form A is non-pathogenic, while the remaining five forms are parasitic on gram plant and produce the same symptoms.

4. The five forms B, C, D, E and F are biologically identical.

5. A discussion of experimental results in relation to the significance of these forms is given and the following conclusions are reached :—

(*i*) Form A does not belong to the species *Ascochyta rabiei* (Pass) Lab.

(*ii*) Forms B, C, D, E and F belong to the species *Ascochyta rabiei* (Pass) Lab. On morphological basis form C can be distinguished from the other forms, but the other forms cannot be distinguished from one another.

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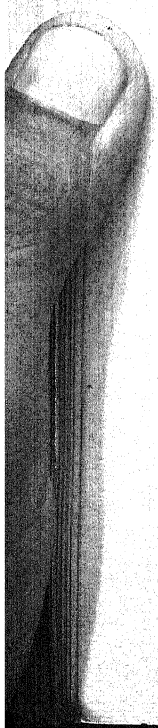

STATISTICAL NOTES FOR AGRICULTURAL WORKERS

NOTE ON THE OPTIMUM SHAPE AND SIZE OF PLOTS FOR SUGARCANE EXPERIMENTS IN BIHAR*

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INTRODUCTION

THE influence of shape and size of plots on the accuracy of comparison is now well-established. Statistical analysis of uniformity trial data has shown that the optimum size and shape for field experiments depends chiefly on the distribution of the fertility gradient in the experimental area, as well as on the nature of the crop under test. Usually there is, however, a best plot size for each crop which is more or less the same for different localities unless there exist marked differences in the nature of the soil. Thus rice experiments in Bengal, Assam and even Ceylon show that the best plot size for variety trials lies between 1/40th and 1/80th acre. In sugarcane trials, Sayer, Vaidyanathan and Iyer [1936] found a plot size of 1/20th acre to be the most suitable for un-irrigated sugarcane; Sayer and Krishna Iyer [1936] observed that the percentage variation can be diminished by increasing the plot size up to 1/27th acre.

Another important point may be noticed here. In unformity trials, the smallest plot size is almost invariably found to be the most efficient. But the smallest plot size is usually inconvenient for agricultural operations. Thus in a sugarcane experiment conducted by Mitra and Phukan in Jorhat (Assam) the highest precision was found for small plots containing only single rows, 13 feet long and separated from one another by 3 ft. furrows.

MATERIAL FOR THE ANALYSIS

The material for the present investigation was obtained from a variety trial experiment with four types of sugarcane, viz., Co 213, Co 210, Co 326 and Co 331, in Bihar. These were laid out in the form of a regular chess-board type of arrangement. The individual plots measured 45 ft. × 45 ft. and contained fifteen rows of plants each 45 ft. long, placed 3 ft. apart. At harvest the rows were divided into 15 units of length, each equal to 3 ft.; and the yield and number of canes in these units were recorded for the whole

* The work was carried out as part of the Sugarcane Research Scheme in Bihar financed by the Imperial Council of Agricultural Research to whom grateful thanks are due.

experiment. Each plot thus gave 225 readings of cane yield and another 225 readings of plant number, so that there were altogether 3,600 observations of cane yields and 3,600 of plant number.

Analysis of concomitant variation

Fisher [1937] has shown that "the precision of an experiment may be greatly increased by equalizing, among the different treatments to be compared, certain potential sources of error". In the present investigation the yield in each row length of 3 ft. was noted along with the number of canes in the strip. An analysis of the number of canes shows that there are appreciable differences from strip to strip. Different parts of the experimental area were clearly exposed to various types of inequalities; in the first place, the soil fertility varied from one part to another; accidental injuries to plants were also of a local character. Assuming therefore that the number of cane is a measure of the inequalities present in the different strips, it is possible to increase the ultimate accuracy considerably. If there is no theoretical objection to comparing the yields of cane based on equal number of plants in the different plots, the method of study appropriate for this purpose is Fisher's Analysis of Covariance. This method has been adopted throughout in this paper. For a proper assessment of its potentialities the analysis of actual yield and of adjusted yield are both given side by side at every stage.

Comparison between varieties

The analysis of variance and covariance and of adjusted yields are given below in Table I.

Table I

Analysis of variance and covariance of the 4 × 4 Latin Square

x = Cane number y = Cane yield

	D. F.	x^2	xy	y^2	Adjusted y^2	D. F.
Rows	3	51,086	70,879	1,04,136	..	..
Columns	3	29,288	40,021	70,188	..	..
Varieties	3	1,44,655	3,63,225	26,01,677	17,13,098	3
Error	6	26,256	38,946	98,165	40,398	5
Total	15	2,51,285	5,13,071	28,74,166	..	..
Variety+error	9	1,70,911	4,02,171	26,99,842	17,53,496	8

The mean values of cane number and of cane yield (actual and adjusted with standard errors) are given in Table II, for each variety.

TABLE II

Mean values of number and yield of cane

Variety	$\bar{x}$	$\bar{y}$	Adjusted $\bar{y}$
Co 213	1,916	3,498	3,726
Co 210	2,169	3,866	3,719
Co 326	2,074	3,648	3,642
Co 331	2,121	4,551	4,475
General mean	2,070	3,891	3,891
Standard error		63·96	44·94
S. E. per cent		1·64	1·15

The standard error of mean yield of a variety based on four replications has been decreased from 1·64 to 1·15 per cent of mean as a result of using the covariance between yield and number of canes. Hence the standard error per plot of size 45 ft. × 45 ft. (containing fifteen rows of plants of length 45 ft. each) is reduced from 3·28 per cent of mean to 2·30 per cent. The problem now is, how far the error of the experiment can be minimised by changing the shape and size of individual plots. For this purpose, we shall take up various shapes and sizes of plots by using different combinations of the unit row strips.

It should be noted here that the estimate of error for comparison of the varieties will not be unbiassed as the design of the main plots was of the systematic chess-board type, but the estimate of error for smaller plot sizes will be quite valid.

COMPARISON OF DIFFERENT SHAPES AND SIZES OF PLOTS

With the material of the present experiment, it is possible to estimate errors for 225 different shapes and sizes of plots by suitably combining the fifteen divisions of each row and the fifteen rows of each plot. In the present report we shall consider the analysis for twenty-five combinations of five row lengths (3, 9, 15, 30 and 45 ft.) with five numbers of rows (1, 3, 5, 10 and 15).

The analysis of variance and covariance for the minimum plot size are shown in Table III.

TABLE III

Analysis of variance and covariance for the unit plot-size "3 feet-one row"

	D. F.	x^2	xy	y^2	Adjusted y^2	Mean y^2	Mean adjusted y^2
Between plots .	15	1,117	2,280	12,774	..	..	..
Within plots .	3,584	46,897	90,038	1,82,580	9,715	50·9431	2·7114
	3,599	48,014	92,318	1,95,354			

The standard error of actual yield per plot of three feet row length is thus 7·14 lbs., and since the mean yield of plot size is 17·29 lbs., the error is 41·30 per cent of mean. The corresponding values for yield adjusted for plant number are 1·65 and 9·54 respectively. The reduction of error is very marked. The standard error in lb. per plot of all the twenty-five combinations are shown in Tables IV and IV (*a*).

TABLE IV

Standard error in lb. per plot for varying plot sizes

Actual yield

No. of rows	Length of rows				
	3 ft.	9 ft.	15 ft.	30 ft.	45 ft.
1	7·14	11·49	14·75	20·96	27·33
3	12·84	23·99	25·06	35·74	56·11
5	17·46	29·41	35·79	52·43	59·78
10	29·64	52·30	68·14	93·72	95·67
15	37·19	59·65	79·89	99·18	127·91

TABLE IV (*a*)

Standard error in lb. per plot for varying plot sizes

Adjusted yield

No. of rows	3 ft.	9 ft.	15 ft.	30 ft.	45 ft.
1	1·65	3·89	3·34	5·42	6·62
3	4·68	14·45	16·53	10·38	35·64
5	3·75	11·86	9·39	11·62	13·55
10	6·14	11·98	16·14	39·50	63·36
15	7·38	15·01	22·62	57·97	89·89

As the plot size increases, there is a more or less proportionate increase in the yields of individual plots. The actual standard error in lb. per plot naturally increases, but if these errors are expressed as percentages of their respective mean yields as shown in Tables V and V (*a*) it is found that the percentage variability between plots steadily diminishes as the plot size is increased. The same thing is more clearly seen from Tables VI and VI (*a*) where the errors have been expressed as percentages of the error of the smallest plot size (three ft. rows).

TABLE V

Standard error per plot in per cent of mean

Actual yield

No. of rows	Length of rows				
	3 ft.	9 ft.	15 ft.	30 ft.	45 ft.
1	41·30	22·15	17·06	12·25	10·54
3	24·70	15·42	9·56	6·96	5·75
5	20·20	11·34	8·28	6·13	4·61
10	17·03	10·02	7·83	5·44	3·66
15	14·34	7·67	6·16	3·86	3·29

TABLE V (*a*)

Standard error in lb. per plot for varying plot sizes

Adjusted yield

No. of rows	Length of rows				
	3 ft.	9 ft.	15 ft.	30 ft.	45 ft.
1	9·54	7·50	3·86	3·17	2·55
3	9·02	9·29	6·19	2·03	1·32
5	4·34	4·57	2 17	1·36	1·04
10	3·53	2·29	1·85	2·29	2·43
15	2·85	1·93	1·74	2·26	2·31

TABLE VI

Standard error per plot as per cent of the standard error of the unit plot (three feet-one row)

Actual yield

No. of rows	Length of rows				
	3 ft.	9 ft.	15 ft.	30 ft.	45 feet
1	100·00	53·63	41·31	29·66	25·53
3	59·81	37·34	23·15	16·85	13·92
5	48·91	27·46	20·05	14·84	11·16
10	41·23	24·26	18·96	13·17	8·86
15	34·72	18·57	14·92	9·35	7·97

TABLE VI (*a*)

Standard error per plot as per cent of the standard error of the unit plot (3ft.—one row)

Adjusted yield

No. of rows	Length of rows				
	3 ft.	9 ft.	15 ft.	30 ft.	45 ft.
1	23·10	18·16	9·35	7·68	6·17
3	21·84	22·49	14·99	4·91	3·20
5	10·51	11·06	5·25	3·29	2·52
10	8·54	5·54	4·48	5·54	5·88
15	6·90	4·67	4·21	5·47	5·59

Thus with plots of one row only, the standard error of actual yield for row lengths of 3 ft. is as high as 41·30 per cent of mean ; as the length is increased, the error diminishes and ultimately for 45 ft. rows, this is only 10·54 per cent of mean. With two rows per plot, there is again a steady diminution of error as the row lengths are increased but the rate of fall is a little lower than in the case of one row plots. A similar decrease will be noticed in all the other cases.

The standard error of adjusted yield also registers similar movement.

Again, if the length of rows is kept constant while the number of rows is increased, there is also a steady decrease of errors, but this is not so marked as that for the increase in row lengths. Justesen [1932], working with potatoes, also observed that a much lower standard error is obtained when the lengths of the plots are increased than when the widths are increased correspondingly. He attributes this to " slicing up " of local differences when long plots are used. so that the differences are more distributed over different plots than is the case when the plots are wider.

EFFICIENCY OF PLOT SIZE

We have seen that the relative variability diminishes as the plot size is increased ; but as the total experimental area cannot be indefinitely increased, the number of replications with bigger plots is necessarily smaller. There are therefore two opposing factors at work in determining the final precision of a given plot size. If the size is bigger, there is a lowering of error and hence an increase in precision ; but as replication is decreased, there is a diminishing precision, inversely proportional to the square root of the number of repetitions. To arrive at the effective error for comparison, therefore, we must multiply the standard error of a given plot size by the square root of the number of unit plots contained in it. This is shown in Tables VII and VII (*a*) expressed as per cent of effective standard error of actual yield for unit plot size.

TABLE VII

Effective standard error per plot as percentage of the effective standard error of the unit plot

Actual yield

No. of rows	Length of rows				
	3 ft.	9 ft.	15 ft.	30 ft.	45 ft.
1	100·00	92·91	92·42	93·80	98·81
3	103·59	112·02	89·66	92·29	93·83
5	109·36	106·35	100·22	104·93	96·65
10	130·37	132·87	134·07	131·70	108·57
15	134·47	124·83	129·13	114·51	119·49

TABLE VII (*a*)

Effective standard error per plot as percentage of the effective standard error of the unit plot

Adjusted yield

No. of rows	Length of rows				
	3 ft.	9 ft.	15 ft.	30 ft.	45 ft.
1	23·10	31·45	20·90	24·25	23·89
3	37·82	67·47	58·04	26·95	21·46
5	23·50	42·87	26·26	23·27	21·82
10	27·02	30·36	31·65	55·40	72·00
15	26·73	31·34	36·44	67·00	83·85

If we compare Tables VI and VI(*a*) with Tables VII and VII(*a*) we find that the larger plots are really not so efficient as they had appeared before. Thus in the case of actual yield if the effective standard error of the 3 feet-one-row plot is supposed to be 100, that for the 45 feet-one-row plot is nearly 99 per cent; but in Table VI, the two standard errors were 100 and 25·53 respectively. The reason for this is very simple: with 3 ft. rows, we may have fifteen replications within the same area as is required for one plot of 45 ft. row. Hence from the point of view of accuracy alone, there is not much to choose between plots of 3 ft. rows and 45 ft. rows.

Many workers have discussed the problem of effective errors in terms of efficiency. Thus Kalamkar [1932] defines " efficiency " as a quantity measuring the total information supplied for a given area. It is calculated by multiplying the variance per plot by the number of ultimate units contributing to the total of that plot and taking the reciprocal. Taking the efficiency of the smallest plot as 100, the efficiency of other plots is shown in Tables VIII and VIII (*a*).

TABLE VIII

Efficiency of plots of different shape and size relative to efficiency of unit plot

Actual yield

No. of rows	Length of rows				
	3 ft.	9 ft.	15 ft.	30 ft.	45 ft.
1	100·00	115·84	117·08	113·66	102·42
3	93·21	79·73	124·40	117·38	114·58
5	83·60	88·42	99·56	90·84	107·10
10	58·82	56·59	55·63	57·66	84·84
15	55·30	64·48	59·97	76·28	70·07

TABLE VIII (*a*)

Effective standard error per plot as percentage of the effective standard error of the unit plot

Adjusted yield

No. of rows	Length of rows				
	3 ft.	9 ft.	15 ft.	30 ft.	45 ft.
1	100·00	53·95	122·04	90·74	93·50
3	37·28	11·72	15·84	73·47	115·87
5	96·62	29·03	77·44	98·63	112·08
10	73·14	57·89	53·27	17·39	10·29
15	74·74	54·36	40·19	11·89	7·59

We notice from Table VIII that there is a marked fall of efficiency as the width of the plot is increased. It will be seen, however, that there is no marked fall of efficiency as the length of the rows is increased. Maximum precision is given by plots containing three rows each 15 ft. long.

But Table VIII (*a*) strangely enough condemns this plot size as one of the least precise. The two plot sizes which have come out consistently efficient in both Tables are each having length 45 ft. and width three rows and five rows. In fact, if smaller plots are difficult to work with, 45 ft.-five row plots with an area of 1/64th acre may be safely recommended for future trials.

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STUDIES ON THE HOT FERMENTATION PROCESS FOR THE COMPOSTING OF TOWN REFUSE AND OTHER WASTE MATERIAL

* II. SOME FACTORS INFLUENCING THE EFFICACY OF THE PROCESS

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(With one text-figure)

IT is well known that the biological decomposition of plant materials is influenced greatly by factors such as the degree of moisture, temperature, the presence of available nitrogen, the degree of aeration, presence of inoculum, etc. These factors also influence the course of decomposition occurring in compost heaps ; and their importance is specially great in the hot fermentation process, since a special feature of this method is the intensive aerobic oxidation concentrated within the first five or six days, after which the mass is packed up anaerobically. It is, therefore, essential that the optimum conditions for rapid decomposition be known and secured even from the beginning.

The experiments described below were carried out under controlled conditions in concrete cisterns coated with cement inside so as to prevent leakage of the liquid constituents. Street sweepings and night-soil were used as compost materials and the influence of the following factors on the rate of composting were examined :—

(1) Use of different proportions of soil, night-soil, leaves and moisture ;

(2) temperature changes during composting ;

(3) variations in the relative periods of preliminary aerobic and subsequent anaerobic fermentation ;

(4) influence of added chemicals, e.g., calcium carbonate, superphosphate, sodium nitrate, ammonium sulphate and calcium cyanamide ;

(5) comparison of the quality of composts prepared in cemented cisterns, underground pits and overground heaps ; and

(6) influence of prior fermentation of street sweepings before the addition of night-soil.

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The composting procedure adopted was as follows :—

Town refuse was screened into two fractions : (1) consisting mainly of soil and ash constituents, and (2) consisting mostly of leaves and other organic matter. These, for the sake of brevity, will hereafter be denoted respectively as the soil and leaves fractions of town refuse. Large stones and other things such as glass, iron pieces, etc., were removed by hand.

The refuse materials used had the following average composition :—

TABLE I

Composition of refuse materials

	Street sweepings screened into		Night-soil (fresh) (per cent)
	Soil fraction (per cent)	Leaves fraction (per cent)	
Total carbon . . .	3·07	30·71	9·30
Total nitrogen . . .	0·24	1·23	1·10
Ash-free organic matter .	6·20	54·30	15·30
Ash	91·30	36·10	4·20
Moisture	2·50	9·60	80·50

(All figures are on fresh basis including moisture.)

The requisite amounts of the soil and leaves fractions of street sweepings and of night-soil were weighed and mixed thoroughly on a brick-lined platform outside the containers, adding the necessary amount of water. The mass was then transferred into cisterns eight-cubic-feet made of concrete and coated with cement inside. A preliminary aerobic fermentation of five to six days was allowed, after which the contents were pressed down and packed anaerobically with a layer of mud-paste and then covered over with more earth. In some cases, the top was coated with a layer of cowdung. Anaerobic fermentation was allowed to proceed for $2\frac{1}{2}$ to 3 months, after which the manure was removed, weighed and representative samples taken for analysis. The remaining bulk of the manure was put back in the same cistern, under suitable cover, till required for manurial trials in pots or plots.

SAMPLING AND ANALYTICAL PROCEDURE

The complete contents of a cistern were removed, weighed and spread uniformly on a cowdung coated platform, so that the soil and leaves were uniformly distributed. A number of small samples were taken at different

spots and these were mixed together to form a composite sample for analysis. The sample was dried in the sun and powdered in a grinding mill before analysis. Carbon was determined by the wet combustion method of the author [Acharya, 1936] and total nitrogen by the Kjeldahl-Gunning method modified to include nitrates [A. O. A. C., 1935]. Ash-free organic matter was estimated by loss on ignition, after making an allowance for moisture present.

(1) Influence of soil on the humification of refuse materials

It was noted in some of the preliminary trials that the addition of soil to the compost heap helped to promote a more rapid disintegration and decay of the refuse materials. With a view to test this point further, trials were carried out with lots of 100 lb. of night-soil and 50 lb. of the leaves fraction of town refuse, and to this were added varying amounts of the soil fraction of street sweepings. Typical results that were obtained are shown in Table II.

TABLE II

Influence of addition of soil on the humification of town refuse

Compost lots : 50 lb. leaves + 100 lb. night-soil	No soil added		100 lb. soil added		200 lb. soil added	
Yield of manure (fresh)	126 lb.		312 lb.		505 lb.	
Yield of manure (sun dry).	58 lb.		150 lb.		260 lb.	
	Percentage analysis of sun dry material	Percentage recovery of constituents originally taken	Percentage analysis of sun dry material	Percentage recovery of constituents originally taken	Percentage analysis of sun dry material	Percentage recovery of constituents originally taken
Carbon . .	23·70	55·76	12·96	70·01	10·41	87·90
Nitrogen .	1·70	57·65	0·88	67·70	0·68	80·74
Ash-free organic matter.	43·70	59·65	23·76	73·18	18·61	88·12

The data show that the addition of soil markedly increases the proportion of the original constituents (e.g. carbon, nitrogen and ash-free organic matter), which is ultimately recovered in the final compost. The increase in the recovery of carbon, for example, from 56 to 70 per cent when 100 lb.

of soil are added to a mixture of 100 lb. of night-soil and 50 lb. of leaves, and the increase of nitrogen recovered from 58 to 68 per cent implies a proportionate increase in the efficiency of the composting process due to the addition of soil provided, of course, the final material is well-formed compost. The addition of household ash contained in the soil fraction of street sweepings also serves to increase the manurial value of the compost in respect of potash and phosphoric acid.

The preliminary observations regarding the beneficial effect of soil in hastening the decay of the refuse materials were again confirmed in the subsequent experiments. This rapid decay was probably due to the combined effect of a number of factors, e.g.,

(*a*) the soil forming an effective inoculum for the introduction of the necessary micro-organisms ;

(*b*) the mineral constituents of household ash, associated with the soil fraction, acting as microbial stimulants ;

(*c*) the retentive capacity of soil for moisture, helping to conserve moisture better in the compost heap and thus promoting better decay and decomposition ; and

(*d*) the soil offering a buffer medium for keeping the reaction steady against the organic acids or ammonia that may be produced.

The smaller loss of organic matter in presence of soil may be explained as being due to the fact that the clay complex of the soil reacts with the constituents of the refuse material to form complexes which are more resistant to microbial decomposition than the original refuse constituents themselves. As such the decay is more physical than chemical. The influence of soil on the rate and course of biological decomposition of plant materials is well worth a detailed study. But it must be noted that the addition of large quantities of soil to the compost will markedly lower the organic matter and nitrogen percentage in the fi al manure obtained ; and the transport charges per unit of carbon and nitrogen will become proportionately higher.

Taking the above facts into consideration, it would appear advisable to add moderate quantities of the soil fraction, say 50 to 100 lb. for every 100 lb. of night-soil and 50 lb. of leaves, in the compost, so as to incorporate the advantages of the presence of soil, but not its disadvantages.

The addition of the soil fraction will also help to make up the shortage of leaf material felt in many municipalities for compost work.

(2) *Influence of different amounts of night-soil*

With a view to determine the maximum quantity of night-soil which could be decomposed with the help of a certain amount of leaf material, so that a manure free from night-soil odour could be ultimately obtained, composts were prepared in cisterns from lots of 50 lb. of leaf material and 100 lb. of soil

fraction, to which varying amounts of night-soil were added (Table III). The composting procedure was the same as that described before.

TABLE III

Influence of different proportions of night-soil

Compost lots : 50 lb. leaves + 100 lb. soil	Added 50 lb. night-soil		Added 100 lb. night-soil		Added 150 lb. night-soil		Added 200 lb. night-soil	
Yield of manure (fresh).	278 lb.		312 lb.		355 lb.		375 lb.	
Yield of manure (sun dried)	146 lb.		150 lb.		162 lb.		171 lb.	
	Percentage analysis of sun dry material	Percentage recovery of constituents originally taken	Percentage analysis of sun dry material	Percentage recovery of constituents originally taken	Percentage analysis of sun dry material	Percentage recovery of constituents originally taken	Percentage analysis of sun dry material	Percentage recovery of constituents originally taken
Carbon . .	10·01	63·35	12·96	70·10	15·01	75·14	17·18	79·36
Nitrogen . .	0·64	66·76	0·88	67·70	0·91	59·17	0·94	52·62
Ash-free organic matter	17·32	61·67	23·76	73·18	26·24	75·65	30·12	80·48

It was observed that composts to which amounts of night-soil greater than 100 lb. (for 50 lb. of leaves *plus* 100 lb. soil) had been added, smelt badly of night-soil, showing that the decomposition was only partial and that the bulk of the night-soil had only remained mechanically stored.

The analytical figures given in Table III show a higher recovery of carbon and of organic matter originally taken, with higher doses of night-soil ; but there is a decreasing recovery of nitrogen above 100 lb. of night-soil. This shows that rapid loss of nitrogen takes place, probably as ammonia, when larger amounts of night-soil are composted with leaves and soil than what correspond to a ratio of 2 : 1 : 2.

(3) Influence of leaves on the composting of night-soil

It is the custom in several localities to make a poudrette of night-soil, by drying it either by itself or in admixture with soil. Under these conditions, there is very little biological decomposition of the night-soil and much of the nitrogen is lost due to liquefaction of the night-soil and the evolution of ammonia. In order to test the advantage of composting night-soil with leaves over the poudrette method, experiments were carried out with mixtures of soil and night-soil to which varying amounts of the leaves fraction of town refuse were added.

It was observed that poudrette, as ordinarily prepared, smelt strongly of night-soil, whereas the composts prepared by the addition of enough leaves and other decomposable organic matter, yielded well humified products without any offensive odour.

The analytical figures obtained are presented in Table IV. They show that the loss of nitrogen is greatest in cases where no leaves are added and also in others where excess of leaves are added. In the former case, the loss may be explained as being due to the absence of a vigorous microbial flora capable of fixing the ammonia evolved from night-soil. In the latter case, the carbon/nitrogen ratio of the compost tended to become narrow and thus resulted in loss of nitrogen.

TABLE IV.

Influence of leaves on the composting of night-soil

	300 lb. soil + 75 lb. night-soil. No leaves added		200 lb. soil + 75 lb. night-soil +25 lb. leaves		100 lb. soil +75 lb. night-soil +50 lb. leaves		100 lb. soil +75 lb. night-soil +100 lb. leaves.	
Yield of manure (fresh)	420 lb.		405 lb.		335 lb.		390 lb.	
Yield of manure (sun dried)	298 lb.		220 lb.		156 lb.		179 lb.	
	Percentage analysis of sun dry material	Percentage recovery of original constituents taken	Percentage analysis of sun dry material	Percentage recovery of original constituents taken	Percentage analysis of sun dry material	Percentage recovery of original constituents taken	Percentage anaylsis of sun dry material	Percentage recovery of original constituents taken
Carbon . .	4·42	81·41	7·42	78·52	11·42	70·18	14·17	62·24
Nitrogen . .	0·31	59·99	0·51	70·13	0·74	69·11	0·72	56·13
Ash-free organic matter	7·84	77·87	14·02	82·47	20·22	70·41	25·77	64·16

It would appear that one part of leaves to two parts of night-soil (in presence of one to two parts of soil) would represent a suitable combination for ensuring rapid composting of the night-soil and at the same time for avoiding much loss of nitrogen.

(4) *Water requirements for composting*

A few experiments were carried out to determine the minimum water supply required for composting purposes by the hot fermentation process. This determination is of special importance in this method, since the mass is anaerobically packed after the first few days and no further additions of water are made during the $2\frac{1}{2}$ to 3 months period of anaerobic storage. In aerobic methods of composting, the moisture is generally maintained at about 50 to 60 per cent level by additions of water from time to time.

The experiments were carried out by adding varying amounts of water (3, 5, 7, 9 and 12 gallons of water) to a mixture of 50 lb. of leaf material, 100 lb. of soil and 75 lb. of night-soil, packed in cisterns according to the procedure already reported above. Assuming the water content of night-soil to be about 80 per cent, the relative proportions of dry matter and water added were as shown in Table V.

TABLE V

Water requirements for composting

	Water added (additional)	Percentage moisture content of watered compost	Remarks
1	*Nil*	29·90	Insufficient humification
2	1 gallon	33·06	
3	3 gallons	38·32	
4	5 gallons	42·81	Satisfactory decomposition
5	7 gallons	46·69	
6	9 gallons	50·07	
7	12 gallons	54·41	Liquid sludge at bottom, showing excess of water, bad sewage odour
8	15 gallons	58·06	

Taken 50 lb. leaves, 75 lb. night-soil and 100 lb. soil.

Dry matter in compost at start = 157·3 lb.

Moisture already present = 67·7 lb.

The moisture requirements under hot fermentation conditions would appear to be fairly well defined. Appreciable deficiency below this level prevented satisfactory rise of temperature in the early stages and hindered further decomposition during the succeeding anaerobic stage, whereas a too liberal dosage of water promoted anaerobic decomposition to such an extent as to form large amounts of ammonia and acid products.

Satisfactory humification of waste materials was secured in the above experiments by the addition of seven to nine gallons of water, *i.e.*, when the moisture content was brought to about 47 to 50 per cent calculated on the final weight of the watered compost. This amount of water was sufficient to bring about rapid aerobic decomposition and rise of temperature in the initial stages and subsequent anaerobic humification. Additions of water more than ten gallons left a good deal of water in the bottom of the cisterns at the end of the decomposition period, when the composts were opened. This happened in spite of the presence of 100 lb. of soil in the compost. The anaerobic decomposition in such cases was well advanced and the mass emitted a bad sewage odour. The liquid sludge at the bottom increased with the amount of initial moisture content above 54 per cent.

These remarks apply mainly to cases where the composting is carried on in brick-lined trenches or cisterns. Where, however, the trenches are not brick-lined, there is great danger of much of the valuable ingredients of night-soil being washed down, if the water supply exceeds an amount equal to that of the dry matter at the start.

It is interesting to compare the water requirement of the hot fermentation process with that of the aerobic methods. In both cases, the initial watering has to be done to bring the moisture content to about 50 per cent (i.e., proportion of dry matter : moisture =1 : 1). In the hot fermentation process, no further additions of water are necessary, since the mass is compacted and anaerobically packed at the end of five or six days. But in the aerobic method, the compost is usually turned over and watered once a fortnight, at least four or five times. The amount of water that has to be added on subsequent occasions will, no doubt, vary with the weather conditions, but it can be roughly estimated that the total quantity of water required for the aerobic process will be three to four times that required for the hot fermentation method. The latter process will, therefore, prove specially valuable in areas of low rainfall or limited water supply.

(5) *Temperature changes during the hot fermentation process*

Daily readings of the temperature of the compost heap were taken at different depths, with the help of a long stem thermometer. The temperature increased up to a depth of 20-25 cm. from the surface, after which it decreased again. Typical variations of temperature over a number of days at three different depths in the compost, viz., 20 cm., 40 cm. and 60 cm. are shown graphically in Fig. I.

In general, it can be stated that the rise of temperature is proportional to the amount of decomposable organic matter present, *e.g.*, leaves. Large amounts of water (above 50 per cent) hindered rapid rise of temperature. The maximum temperature (65-70°C.) was usually reached in about three to five days, after which the temperature slowly fell. The initial rise of temperature was conserved better in the system of packing in trenches adopted in the hot fermentation process than in the aerobic heaps overground, which permitted access of air from the sides and consequent cooling of the mass.

The high temperatures of 65-70°C reached in the initial stages served to destroy pathogenic organisms and weed seeds present in the compost heap. The fly larvae generally came to the top after the first day or two and were later destroyed when the heaps were covered over anaerobically with a layer of earth. The effectiveness of the destruction was shown by the complete absence of fly breeding, which is a common feature of other systems of composting night-soil and is a source of danger to public health in the surrounding area. Further, when the hot-fermented manure was later applied to land, the amount of weeds that developed was much less than when farmyard manure, as ordinarily obtained, was used.

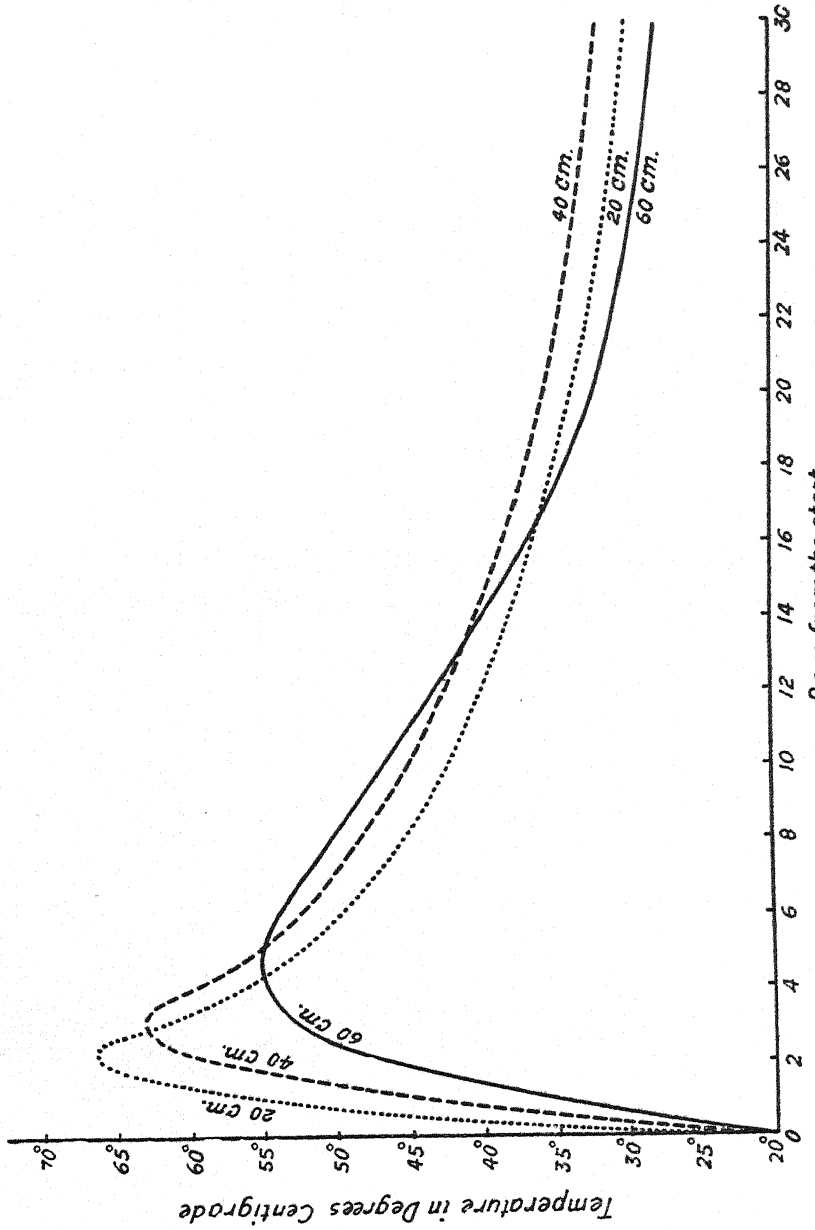

FIG. 1. Temperature changes at different depths of the compost heap

The initial high temperatures last for about four or five days after which the curves tend to come down. It is at this stage that the heaps are usually packed up anaerobically. Such packing helps to maintain the temperature at a high level for a long period and thus promotes thermophilic anaerobic decomposition.

(6) Periods of preliminary aerobic and subsequent anaerobic decomposition

A consideration of the temperature curves given above would show that very rapid chemical decomposition takes place in the first few days and the vigour of the reaction abates considerably after four or five days. In order to examine whether a similar picture is shown by chemical analysis at different stages of the decomposition, experiments were carried out with lots of 50 lb. leaf-matter, 100 lb. soil fraction and 75 lb. of night-soil. The recoveries of carbon and nitrogen expressed as percentages of the amounts originally taken, are given in Table VI.

TABLE VI

Period of preliminary aerobic treatment

Period of preliminary aerobic treatment (days)	Temperature (°C)	Percentage recovery of carbon	Percentage recovery of nitrogen
1 . . .	55	92·1	89·4
2 . . .	65	84·8	82·1
3 . . .	62	79·3	77·6
5 . . .	54	75·6	74·3
7 . . .	50	74·4	72·1
10 . . .	44	72·9	71·6
15 . . .	40	70·7	69·4

The figures show that chemical analysis confirms the temperature indications, *viz.*, that the bulk of the decomposition takes place within the first week and that the subsequent change proceeds more slowly. But, since the mass is compacted free from further access of air after the first five or six days, the subsequent anaerobic change may be expected to be much slower still and to consist mainly of a process of humification, without appreciable loss of carbon or of nitrogen.

This was tested by a further series of compost lots of 50 lb. leaf-material, 100 lb. of soil fraction and 75 lb. night-soil, which were kept aerobic for five days and then were packed anaerobically in cisterns. The cisterns were opened at periodical intervals, the contents weighed and representative samples taken for analysis. The data obtained are presented in Table VII.

TABLE VII

Period of anaerobic packing

Period of anaerobic packing after preliminary aerobic treatment for five days	Percentage recovery of carbon	Percentage recovery of nitrogen
Nil	74·4	72·1
1 month	72·1	70·6
2 months	70·9	70·2
3 months	70·1	69·6
4 months	69·6	69·1
6 months	69·0	68·4

It may be rather misleading to interpret the data given above as indicating that it is unnecessary to subject the mass to the subsequent process of anaerobic decomposition. Though this second stage does not result in any appreciable net loss of carbon or nitrogen, it does signify a period of important anaerobic transformations proceeding in the mass whereby the compost is well humified and its nitrogen is rendered more easily nitrifiable and available to the plant. A period of two to three months' storage is necessary before the manure can be considered ready for application to land.

(7) *Influence of the addition of chemicals on the course of decomposition*

It is well known that the biological decomposition of plant materials is greatly influenced by the reaction of the medium, by the presence of nitrogenous starters such as calcium cyanamide, ammonium sulphate or sodium nitrate and by the addition of chemicals such as superphosphate, potash, etc.

Experiments were carried out on the usual lines with compost lots of leaves, soil and night-soil, in order to test the effect of the addition of the above substances. The reaction was sought to be maintained (*a*) on the neutral or slightly alkaline side by addition of lime ; and (*b*) on the acid side by the addition of molasses, which, under anaerobic conditions rapidly ferments to organic acids.

No beneficial effect was noticed due to the addition of lime, probably because the soil fraction used in the compost contained already a good amount of household ash, which is alkaline. Further, the night-soil present in the compost tends to become alkaline due to liberation of ammonia, which serves to neutralise any organic acids formed in the anaerobic stage. The soil also acts as a good buffer medium.

The addition of nitrogenous starters such as ammonium sulphate and calcium cyanamide had also no effect, showing that night soil was a sufficiently powerful starter by itself and does not stand in need of supplements. Superphosphate had also no beneficial effect, probably due to the fact that the ash constituents present in the soil fraction supplied the necessary amount of phosphoric acid for microbial activity.

Sodium nitrate and molasses, however, yielded interesting results. In the former case, the recoveries of both carbon and nitrogen were markedly decreased (Table VIII). It is well known that sodium nitrate, in addition to being denitrified under anaerobic conditions, is a good source of oxygen for the oxidation of other substances present along with it under such conditions [Acharya, 1935]. It is, therefore, not surprising that in addition to losing the whole of its own nitrogen, it brings about the loss of nitrogen already present in the night-soil added to the compost. It is doubtful whether sodium nitrate, on account of the ease with which it can be leached out and other disadvantages, could be recommended as a starter for composting work under any conditions even including aerobic.

TABLE VIII

Influence of added substances on composting

Substance added	Percentage recovery of carbon	Percentage recovery of nitrogen
(1) Control without any addition	70·18	69·11
(2) Added 10 lb. $NaNO_3$	55·62	39·59
(3) Added 40 lb. molasses	59·14	80·42

(Compost lots ; 50 lb. leaves ; 100 lb. soil and 75 lb. night-soil.)

In this connection, it may be noted that any nitrate present in the compost at the start is likely to behave similar to sodium nitrate and is likely to be lost in the hot fermentation method. It is, therefore, advisable to see that the initial period of aerobic decomposition is just enough to oxidise the easily decomposable constituents of the compost and is not prolonged so as to form nitrate. Under the conditions of the composting procedure described above, where the compost heap is packed up anaerobically when the temperature falls from 65° to 50 °C., it is doubtful whether nitrification (optimum temperature 30-35°C.) would have begun. Hence no loss of nitrogen need be feared on this account in the present method.

In the case of molasses, the figures show a lower recovery of carbon but a higher recovery of nitrogen originally taken to start with. This can be

explained as due to the fact that (*a*) molasses, in addition to being easily oxidised itself, serves as a source of energy for the oxidation of other substances; hence the recovery of carbon is low; and (*b*) molasses yields a good amount of acids which serve to fix the nitrogen of the night-soil, which otherwise would have been lost as ammonia; hence the recovery of nitrogen is higher than in the control. It is doubtful, however, whether the use of molasses for this purpose would be a practical proposition on account of its cost and transport charges.

(*8*) *Comparison of different methods of packing the hot fermentation compost*

The experiments described above were all carried out under controlled conditions in concrete cisterns which were cement-coated to prevent any possible seepage of the liquid contents. But since, on the large scale, the preparation of such cisterns would be uneconomical for purposes of composting, other types of containers were tried which would be cheaper.

It has already been stated that the temperature of the composting mass is conserved better in underground trenches or cisterns than in heaps overground, which are exposed to air on all sides.

The following types of packing the compost were compared:—

(1) Concrete, cement-coated cisterns 2 ft. × 2 ft. × 2 ft. of the type used in the previous experiments; these were over ground.

(2) Trenches, dug in the ground, 3 ft. × 3 ft. × 20 ft., which were lined with brick and plastered.

(3) Similar trenches, underground, which were not brick-lined or plastered.

(4) Heaps overground on brick-lined and plastered platforms.

(5) Heaps over ordinary ground which was hardened and coated with a thick layer of cow-dung, but without any brick-lining or plastering.

In all cases, the proportion of the leaf-matter : soil : night-soil was 50 : 100 : 75, though the actual amounts used varied with the size of the containers. At the end of six days, when the temperature was falling, the heaps were compacted and covered over with a layer of earth and plastered over with cow-dungmixed with water. The heaps were re-opened at the end of three months, weighed and analysed.

The recoveries of carbon, nitrogen and ash-free organic matter are shown in Table IX.

TABLE IX

Comparison of different methods of packing the hot fermentation compost

Percentage recovery of amounts taken at the start	Concrete cisterns cement-coated (over-ground)	Trenches brick-lined and plastered (under-ground)	Trenches without brick-lining (under-ground)	Heaps over-ground on brick-lined plat-forms	Heaps over-ground without brick-lined plat-forms
Carbon	70·18	76·96	58·12	62·81	51·13
Nitrogen	69·11	74·16	59·22	64·62	54·57
Ash-free organic matter	70·41	78·81	57·58	61·46	53·50

The figures show that the highest conservation of carbon and nitrogen is obtained in brick-lined trenches underground, followed respectively by systems of packing 1, 3, 4 and 5 mentioned above. The low value obtained for heaps overground, without a brick-lined platform, was, no doubt, due to seepage of the constituents into the underground layers, especially when there was heavy rainfall. The heaps on brick-lined platforms analysed better, but these were poorer in carbon and nitrogen than the composts made in brick-lined trenches, probably due to continued aeration of the overground mass in the later ' anaerobic ' stage, in spite of the mud covering.

It was observed during the experiments that the manure prepared in the underground trenches—both brick-lined and without brick-lining—had humified better and contained larger quantities of fungal matter than the composts prepared in concrete, cement-coated cisterns overground. This was probably due to the fact that the much bigger mass of compost packed in the former case produced a better rise of temperature and a more advanced stage of humification than in the latter.

The composts prepared in underground trenches, without brick-lining, lost, no doubt, some of the nitrogenous components due to leaching (since the nitrogen recovery is poorer than in trenches with brick-lining), but they were better than the composts prepared in heaps overground. The adoption of underground trenches without brick-lining is, however, limited to areas where the water-table is not too high, since in the latter case, the loss of nitrogen will be specially heavy. In cases where the water-table is high, it would be essential to brick-line and plaster the sides of the trenches ; otherwise, overground cisterns or overground heaps on brick-lined platforms would have to be adopted.

(9) Effect of prior fermentation of leaf material before addition of night-soil

It will be noticed from the data presented in the above tables that a good percentage of nitrogen (from 25 to 30 per cent) is lost while composting night-soil even by the hot fermentation process. It was felt that the loss might have taken place as ammonia, on account of the rapid liquefaction of night-soil. Attempts were made to secure a higher conservation of nitrogen by subjecting the leaf material to a preliminary anaerobic decomposition so as to form organic acids which might later help to fix the ammonia evolved from night-soil.

The experiments were conducted with lots of 50 lb. screened leaves fraction *plus* 100 lb. of soil fraction which were sprayed on with water to bring the moisture level to 50 per cent. and allowed to decompose in covered cisterns for four or five days. At the end of this period, 75 lb. of night-soil were added to each lot and then one series was kept aerobic and turned over once in ten days while the other was subjected to the hot fermentation process as described before.

In order to increase the amount of acids formed during the initial anaerobic decomposition, another series was run on the above lines by the hot fermentation procedure, with the difference that 10 lb. of molasses were added to the leaves fraction at the start.

The recoveries of carbon and nitrogen obtained in the different cases are shown in Table X.

TABLE X

Influence of previous fermentation of leaves

	Lots of 100 lb. leaves, 50 lb. soil and 75 lb. night-soil	Aerobic process		Hot fermentation process	
		Percentage recovery of carbon	Percentage recovery of nitrogen	Percentage recovery of carbon	Percentage recovery of nitrogen
1	Without preliminary fermentation of leaves	51·42	56·28	70·18	69·14
2	With preliminary fermentation of leaves	47·61	52·14	68·61	70·22
3	Preliminary fermentation of leaves with 10 lb. molasses	42·48	60·26	62·38	77·46

The figures show that there is no appreciable gain due to the preliminary fermentation of leaf material. This might have been due to the fact that not much of acidity was produced during the decomposition of leaf material, which was already rich in nitrogen (1·23 per cent). The recovery of nitrogen

is better where molasses had been added at the start, but, as has been already observed, the addition of molasses for the purpose will not be an economic proposition. Moreover, the addition of molasses serves to depress the recovery of carbon on account of its functioning as a readily available source of energy for oxidation purposes.

Summary

Experiments carried out under controlled conditions with street sweepings and night-soil in order to examine the influence of various factors on composting the above materials by the hot fermentation process showed :

1. The addition of soil—especially the screened soil *plus* ash fraction of street sweepings—to a mixture of night-soil and leaves, promoted a more rapid disintegration and decay of the compost material and decreased the loss of nitrogen and of organic matter. Addition of large amounts of soil, however, decreased the manurial value of the compost.

2. In the absence of leaves and other easily decomposable fractions of street sweepings, the mixture of night-soil and soil decomposes slowly and most of the night-soil remains as such in the dry condition. The resulting poudrette of soil and night-soil smells badly of night-soil.

3. An optimum mixture for composting purposes would consist of one part by weight of leaves fraction of street sweepings, one to two parts of the soil fraction and two parts of night-soil.

4. An initial moisture content of about 50 to 55 per cent in the compost heap at the start is optimum, and further additions of water are unnecessary in the hot fermentation process. A moisture level less than 40 per cent results in insufficient humification and a level higher than 55 per cent leads to the formation of liquid sludge at the bottom and loss due to seepage.

5. The water requirements of the hot fermentation process are much less—about a third or fourth—than for ordinary aerobic methods of composting.

6. Temperature measurements made at different depths and on different days showed that the temperature rapidly rose from the second day and reached a maximum of about 65° to 70°C. on the third or fourth day, after which it slowly fell. The temperature increased from the outer layers inward till a maximum was reached at a depth of about 20 cm. in the earlier stages and at a depth of about 40 cm. in the later stages of composting.

7. The bulk of the decomposition in the hot fermentation system takes place during the preliminary aerobic treatment of five or six days ; the loss in weight in the subsequent period of anaerobic decomposition is very small.

8. No beneficial effect was noticed due to the addition of lime or superphosphate to the compost of night-soil and street sweepings. The addition of nitrogenous starters, such as calcium cyanamide, sodium nitrate or ammonium sulphate, was also unnecessary where night-soil was present, and only served

to increase the total loss of nitrogen. Sodium nitrate, on account of its oxidising action, also served to increase the loss of organic matter. The addition of molasses served to decrease nitrogen loss, but increased the loss in organic matter.

9. A comparison of different containers for carrying out the hot fermentation method, *viz.*, (*a*) concrete, cement-coated cisterns ; (*b*) underground trenches which are brick-lined and plastered ; (*c*) underground trenches without brick-lining ; and (*d*) overground heaps on un-brick-lined platforms, showed that there was considerable loss of nitrogen and of carbon, apparently due to seepage, in cases where the trenches or platforms were not brick-lined. Between overground heaps and underground trenches, the latter promoted more ' uniform ' decomposition of the material and conserved the manurial constituents better.

10. Prior fermentation of street sweepings anaerobically for a few days before adding night-soil did not materially decrease the loss of nitrogen. The addition of molasses, however, to the fermenting street sweepings, decreased the loss of nitrogen when night-soil was subsequently added, but the gain was not worth the additional expense involved.

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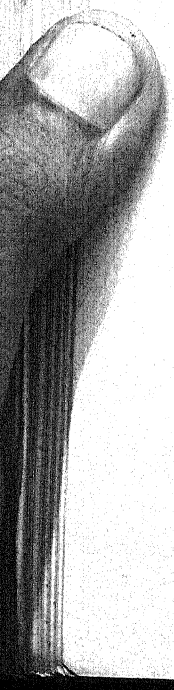

RELATION OF GROWTH TO FRUIT BEARING IN MANGOES

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I. Introduction

IRREGULAR or biennial bearing in the mango is a well-known phenomenon and hardly needs any explanation. As far back as 1590, Abul Fazal, author of the *Aini-Akbari*, an encyclopedic work, while giving an account of this fruit, mentioned that some trees yielded in one year a rich harvest and less in the next, while others yielded for one year and produced no fruit at all in the following year. Similarly Hartless [1913] writes that he had never heard of any variety of mango that flowered normally every year. Burns and Prayag [1920] have mentioned that the record of the mango crop from 1908-16 taken from Ganeshkhind Botanical Gardens on the whole confirmed the belief that the mango tree bears a heavy crop only in every alternate year and that in the intervening year the crop is poor.

The bearing habit of the tree in alternate years is so regular that the 'off' and 'on' year can be anticipated almost with certainty, unless, of course, it is altered by some unforeseen circumstances, such as unfavourable climatic conditions—frost, hailstorm or untimely rain at the time of flowering or incidence of some disease or pest. The problem is of great economic importance to the fruit growers as in the 'on' year, due to the abundance of the crop, the markets are glutted with the fruit and in consequence the price realized is low and unremunerative whilst in 'off' years the growers are confronted with very little crop to offer for sale when the prices are high. To obtain, therefore, a profitable income from a mango orchard it is very essential that the trees during their entire productive life should produce a reasonably regular crop each year. To attain this object it is very necessary to understand the growth behaviour of a tree as influenced by climatic conditions and cultural practices and correlate such growths to fruit bud formatiom. Once a certain type of growth, its behaviour during the growing season as well as its relationship with productivity or otherwise, are all clearly understood, this should serve as a guide to a grower for securing a desirable type of growth by adopting cultural practices most suited for the purpose. Growth response which a tree gives, although morphological in nature, is nevertheless an expression of the condition in the cell or tissue brought about by external and internal factors and can serve as a convenient and simple guide for predicting the future behaviour of the plant.

As growth behaviour of mango tree is rather peculiar, a brief account of the same under Lyallpur conditions would be desirable. Unlike many other fruits the growth in mango in the course of a season is periodic rather than continuous, i.e. successive periods of growth alternating with quiescence. These periodic growths are usually termed as flushes. The number of flushes occurring during the growing season in a year as well as the date on which they occur and the period over which they extend varies with the variety, climatic conditions, cultural practices, age of the tree and the amount of the crop borne by the tree. Yet under Lyallpur conditions in Langra mango growth initiation takes place practically during each month commencing from April and ending with August and consequently as many flushes as five may be

produced during the growing season. Each flush, after it is initiated, grows for some time, stops and breaks out again till the growth finally ceases. The amount of growth produced by each flush in different years is again very variable, in some years it is more while in others it is less.

The flower panicles are borne terminally on the last season's growth only and consequently once a shoot has born flowers or fruit it does not grow from the terminal bud, but the growth, if any, is made from the lateral buds. Again seasonal elongation of growth has a direct bearing on fruit bud differentiation but no work of the type has so far been done on this fruit. Numerous workers from time to time have studied the growth behaviour to fruitfulness in other fruit trees. Gourley [1915], Edminster [1917], Heinicke [1917], Roberts [1920, 1926], Maney and Plagge [1921], Hooker [1925], Dorsey and Knowlton [1925] and Swarbrick [1928], working on apples, have pointed out the importance of individual shoots or spurs in relation to fruit bud formation. All these investigations emphasize the importance of the relationship between growth characters and fruiting potentialities in applied plant physiology in horticulture.

In order to study the relationship between various kinds of growth and productivity and to elucidate some of the growth conditions responsible for alternate bearing in the mango tree, a good deal of data has been collected during the years 1932 to 1938. It may be mentioned that due to lack of suitable material, the investigation could not cover several other phases, such as manuring, irrigation, ringing, pruning and carbohydrate-nitrogen ratio and its bearing on the vegetative growth, fruit production, etc., but it is gratifying to note that the extensive data so far collected have given some definite and valuable information in regard to the behaviour of various types of growth and their relation to fruiting in the following year, as well as the cause of alternate bearing and how this habit could be averted. The aim of the present paper is to deal with the findings of the investigations to date since the inception of the experiment.

II. Procedure and material employed

This paper deals with the investigations extending over a period of six years, the data having been collected by several workers. The investigations started in 1932 when the data were collected by K. C. Naik. During 1933-34 performance records were taken by K. C. Naik and Abdul Aziz Khan. In 1934-35 growth measurements were taken by Pritam Singh Diol, but owing to the occurrence of frost in January 1935, the fruit buds were totally damaged and consequently the data could not be utilized. In 1935-36 the growth performance and flowering records were taken by Abdul Aziz Khan and in subsequent years by Sangat Singh.

Full-grown grafted Langra mango trees, apparently uniform with regard to their size, vigour and productivity, were selected for this investigation in the Mango and Date Avenue at the Punjab Agricultural College, Lyallpur.

The trees included in the trial have all been raised by enarching over mango seedlings of unknown origin. This, no doubt, introduces an element of uncertainty as regards rootstock, but as all grafted mangoes are almost exclusively propagated by this method, it is believed that the results obtained would fairly represent the conditions in mango plantations in the Punjab.

These trees are growing under practically uniform conditions of soil since their planting in 1913. None of these have ever been pruned and in other respects also they have been receiving fairly uniform treatments. From six Langra mango trees 134 and 120 shoots, initiated on the previous year's growth in the months of April and June 1932, respectively, were selected at random. The shoots at the top of the trees being very high and not within easy reach of the recorder were not included during this year, though in subsequent selection of new shoots this was also done. These were numbered and labelled and designated as April and June flush shoots. Growth measurements from the previous year's leafscar of each individual shoot were started as soon as any noticable enlargement of the growth took place and were recorded every week in halves of centimetres throughout the growing season till the period of quiescence when the growth indicated no further enlargement. In the spring of 1933, behaviour of individual shoots with respect to their bearing or non-bearing was noted and the means of the seasonal elongation growth of both bearing and non-bearing shoots under each flush were computed. Again the performance records of these shoots with respect to their growth at monthly intervals were taken in the growing season of 1933 and the bearing and non-bearing shoots out of these were again recorded separately in the spring of 1934 and their means of seasonal elongation compiled on the lines explained already. It would appear, therefore, from the above that the performance records of the shoots selected in 1932 were continued up to the spring of 1934 after which period these were discarded on account of injury caused to them by frost in 1935.

In the spring of 1935 only one tree was taken upon which shoots initiating at different times of the growing season were selected at random and these shoots were designated as April flush, May flush, June flush, July flush and August flush shoots according to the month in which they started to grow. The number of shoots belonging to July and August flushes was rather small but this could not be helped as these were the only shoots in their respective categories produced by the entire tree. Special precautions were taken to select these flushes on the previous year's growth, to eliminate the chance of selecting shoots, in the later part of the growing season, on the earlier flushes and designating these wrongly. As a matter of fact, they may be the continuations of the flushes produced in the beginning of the growing season. The performance records of these shoots were continued to be taken up to the spring of 1938 on the lines already explained.

In addition, 120 flowering shoots were selected on the same tree in the spring of 1935 out of which 20 were deblossomed intentionally early April, while seven others were accidentally deblossomed. The performance of the deblossomed shoots was maintained till the spring of 1938.

In 1937 fresh shoots were selected from a single tree again, this tree being different from the one included in the trial in the spring of 1935. In the spring 100 shoots each of April, May, June, July and August flushes were labelled and their growth performance continued at fortnightly intervals till the shoot elongation had finally ceased. In 1938 their behaviour as to fruiting was studied and the data compiled on the lines detailed above.

Before closing this section of the paper, it is necessary to explain one point. Some may object to the inclusion of only one tree in the investigation after the year 1934. With regard to this, the attention of the reader is called to the importance of the ' build up ' of the tree emphasized by R. G. Hatton [1930, 1931] who, after a long series of experiments on the standardization of apple rootstocks, has shown the necessity of producing the rootstocks by vegetative means to reduce errors in the field experiments. To be sure of the ' build-up of the tree only one tree was selected, in which case there being only one stock and one scion, the element of uncertainty with regard to the material was eliminated. Of course the maximum number of shoots of representative type available on the tree in each category were included in the investigation and wherever possible not less than 100 shoots were selected in each category to start with, although, of course, later on, on account of sub-classification of the shoots according to their behaviour with regard to fruiting, the number in each group was reduced.

The nature of the work does not appear to demand the application of any statistical method to determine significant differences in the results as most of the data accumulated during the period under study relate primarily to the periodicity and cessation of growth of shoots of bearing and non-bearing type. The general behaviour and consistency of the main results shown throughout the investigations is of sufficient significance to justify the discussion of the results without the application of any such method. However, in 1932 and 1933 when the trees under observation were six representing six replicates, the method of analysis of variance advocated by Fisher [1934] was applied on Tables II, IV and VI in the appendix to see whether the results were significant. From each table variances due to different variables were separated and standard error of the mean and significant difference calculated from the variance due to error. Standard errors of the means and significant differences were calculated and shown along with the tables.

III. Presentation of data.

In connexion with the periodicity or seasonal elongation of the shoots and their subsequent behaviour in regard to flowering or non-flowering in the following spring, the terms ' would-be flowering shoots ' and ' would-be non-flowering shoots ' are frequently used. In order to avoid any misunderstanding, it may be mentioned that ' would-be flowering shoots ' would refer to those shoots that flowered in the following spring and ' would-be non-flowering shoots ' were those that did not flower in the following spring.

1. Periodicity of extension growth, 1932

(*a*) *April flush*

1. Actual mean extension growth.—Data regarding the mean total extension growth in centimetres per shoot in 1932 of April flush shoots that flowered or remained barren in the following year is given in Table I and shown graphically in Fig. 1 (*a*).

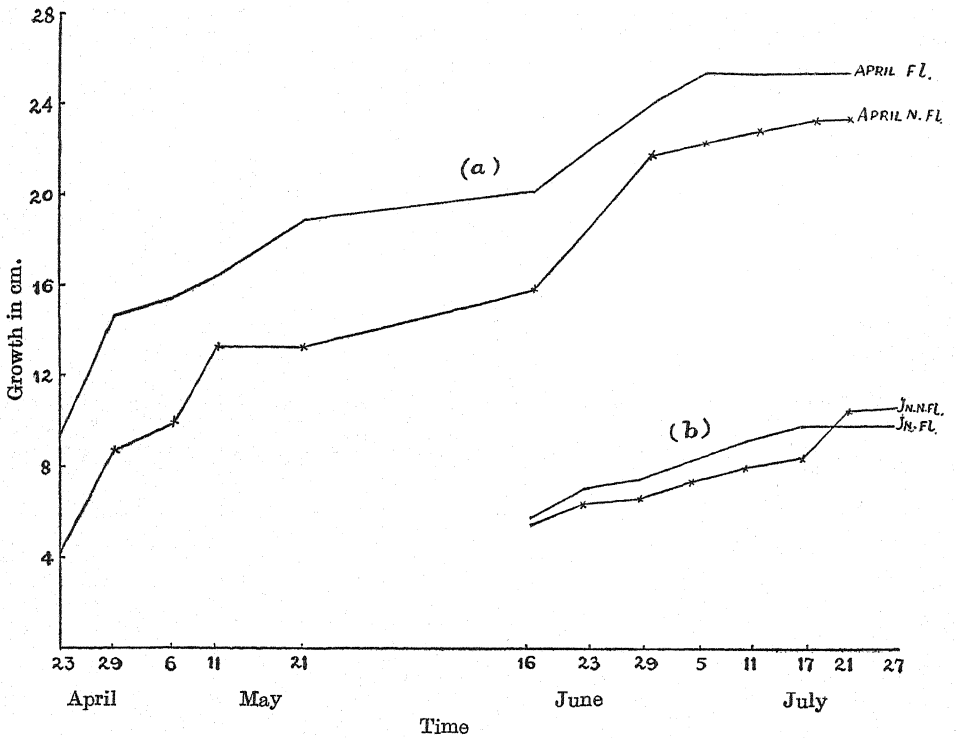

Fig. 1. Mean extension growth in 1932 of 'would-be flowering' and 'would-be non-flowering' shoots of April and June flushes

Fig. (*a*) 1 shows that during the last week of April the growth rate was high in the case of both 'would-be flowering' and 'would-be non-flowering' shoots after which till the beginning of May the growth slowed down in both the cases. From 6th to 21st May there was a gradual increase in the growth of 'would-be flowering' shoots, whereas in 'would-be non-flowering' ones the growth was rapid from 6th to 11th May, after which it stopped altogether for about ten days. From 21st May to 16th June there was a gradual increase in both the shoots after which another stimulus was observed till the end of June in both the 'would-be flowering' and 'would-be non-flowering' shoots. From the beginning of July till the end of the growing season, growth slowed down in the 'would-be non-flowering' shoots whereas in the others it stopped altogether.

2. Calculated growth on percentage basis.—Data in Table I were re-calculated on the basis of percentages on the final length of the shoots at the end of the growing season and shown in Table II.

Table II shows that from the beginning of the growing season till the middle of June, the percentage amount of growth produced by the shoots that flowered subsequently was significantly higher than those that remained barren. After this till the end of the growing season there was no significant difference between the percentage growth of shoots of both the categories, although the figures in the case of ' would-be flowering ' shoots remained comparatively high.

(*b*) *June flush*

1. Actual mean extension growth.—Data regarding the mean total extension growth in centimetres per shoot in 1932 of June flush shoots that flowered or remained barren in the following year is presented in Table III and shown graphically in Fig. 1 (*b*).

Fig. 1. (*b*) shows that out of the shoots of June flush those that subsequently flowered or remained barren there was practically no difference in the rate of their extension growth from the beginning of the growing season till the middle of July, after which the growth ceased altogether in the case of ' would-be flowering ' shoots whereas the ' would-be non-flowering ' shoots grew rapidly for a week and slowed down later on at the end of the growing season.

2. Calculated growth.—Data from Table III were re-calculated in percentages on the final length of the shoots and presented in Table IV.

Table IV shows that, from the beginning of the time the growth started till the middle of July, the percentage amount of growth in the ' would-be flowering ' shoots was significantly higher than the ' would-be non-flowering ' ones. After the middle of July the percentage growth of both the types of shoots was practically at the same level.

2. Periodicity of extension growth, 1933

(*a*) *Non-bearing shoots of April and June flushes*

1. Actual mean extension growth.—Data regarding the mean total extension growth in centimetres per shoot in 1933 of shoots that flowered or remained vegetative in the following year (1934) is given in Table V and shown graphically in Fig. 2.

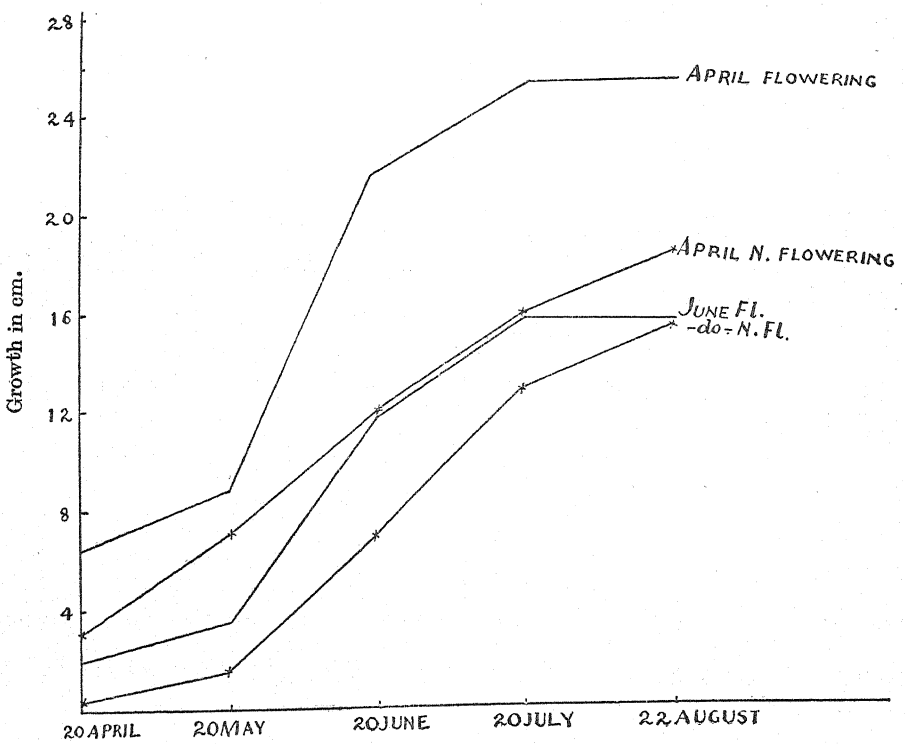

FIG. 2. Mean extension growth in 1933 of 'would-be flowering' and 'would-be non-flowering' shoots of April and June flush shoots non-bearing in 1933

In Fig. 2, while comparing the growth curves of the April flush it is evident that greater amount of growth was produced by shoots that flowered in the subsequent year than those that remained barren from the 3rd week of May to the 3rd week of June after which period both the types of shoots grew practically at the same rate for a month. Towards the end of the growing season for about a month the shoots that flowered in the following spring had stopped growing, whereas during this period the others continued growth at a rapid rate.

With regard to the June flush shoots, both kinds of shoots grew practically at the same rate during the first month of their growing season. During the 2nd month, however, the 'would-be flowering' shoots grew at a more rapid rate than the 'would-be non-flowering' ones. During the next month the 'would-be non-flowering' shoots grew more than the others and during the last month of the growing season the 'would-be flowering' shoots stopped growing altogether while the 'would-be non-flowering' ones continued growth.

2. Calculated growth.—Data from Table V were re-calculated in percentages on the final length of shoots and presented in Table VI.

Table VI shows that the percentage amount of growth produced by the ' would-be flowering ' shoots of April flush was more than the ' would-be non-flowering ' ones throughout the growing season, though, during the first month of the growth, the difference was not significant. In the case of the June flush the ' would-be flowering ' shoots produced significantly more growth than the ' would-be non-flowering ' ones throughout the growing season.

If comparison is made between ' would-be flowering ' shoots of the April and June flushes it is evident that growth produced by the April flush was significantly more than the June flush till the last week of June but during the end of the next month the shoots in both the flushes were at the same level. The behaviour of 'would-be non-flowering ' shoots of both the flushes was also the same.

(b) Bearing shoots of April and June flushes

1. Actual mean extension growth.—As is clear from Table VII, out of 50 shoots of the April flush and 39 of the June flush, all of which were bearing fruit, only 15 in the former and two in the latter made extension growth during the same year, and out of these only two in the former case and none in the latter flowered in the following spring (1934). The data regarding the mean total extension growth in 1933 of bearing shoots that flowered or remained vegetative in the following year is given in Table VII and shown graphically in Fig. 3.

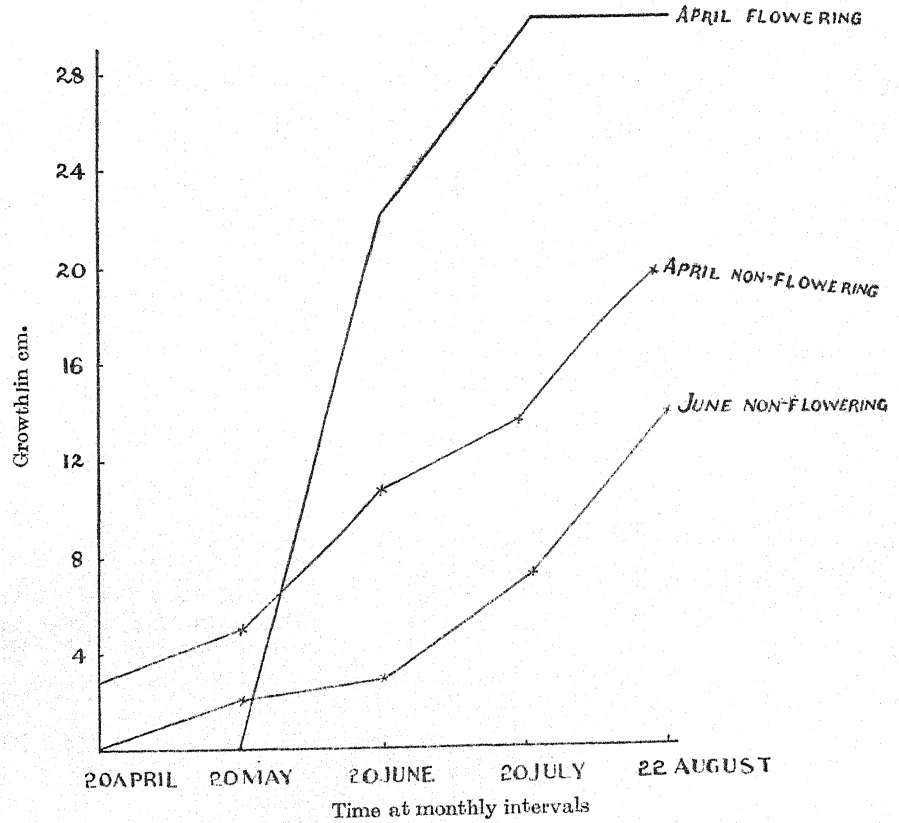

FIG. 3. Mean extension growth in 1933 of ' would-be flowering ' and ' would-be non-flowering ' shoots of April and June flushes bearing in 1933

Fig. 3 shows that the bearing shoots of the April flush, which also flowered in the following spring (1934), made very rapid growth from May to June (1933) and for another month continued growth at a slower rate and ceased growing altogether towards the end of July. On the other hand those bearing shoots of the April flush which did not flower in the subsequent year (1934) continued growth till late in the season of 1933 with no indication of rapid growth between 20th May and 20th June.

No shoot of the June flush flowered and consequently no growth curve for these is plotted in the figure. The ' would-be non-flowering ' shoots of this flush if compared with ' would-be non-flowering ' shoots of the April flush show that their growth curves are similar, especially during the later part of the growing season.

2. *Calculated growth.*—From Table VII the mean growth in percentages on the final length of shoots was calculated and is shown in Table VIII.

In Table VIII no significant differences were calculated owing to the paucity of shoots under each category, though it is quite evident that during the month of June and July the growth of 'would-be flowering ' shoots of the April flush was more than the ' would-be non-flowering ' ones.

3. GROWTH PERFORMANCE AND FLOWERING RECORDS OF SHOOTS FROM 1933-34

(*a*) *Actual growth and flowering record of shoots selected in 1932*

Data regarding the growth and flowering record, from 1932-34 of April and June flush shoots selected in 1932 are presented in Table IX.

Columns 1 and 2 of Table IX give the name of flush and number of shoots selected in each flush respectively. Column 3 gives the number of shoots that flowered or remained barren out of the shoots in column 2. Column 4 gives the number of shoots that grew out of those in the previous column. Column 5 gives the number of shoots that flowered or remained vegetative out of shoots in the previous column. This table shows two things of great interest, firstly that the shoots that flowered were out of those shoots that made extension growth in the preceding year and secondly the flowering shoots during their year of bearing grew in less number as compared to non-flowering ones. The latter point is brought out more clearly in Table X.

(*b*) *Percentage number of shoots flowering and growing*

Data from Table IX are re-calculated on percentage number of shoots growing and bearing in each year and presented in Table X.

In column 2 of Table X the shoots of each flush selected are assumed as 100. In the 3rd column the percentage of shoots flowering and non-flowering out of column 2 is given. In column 4 percentage of shoots growing out of the previous column is presented, while in the last column the percentages given are calculated on the number of shoots in column 3.

Table X shows that out of 100 shoots growing in 1932 in each flush, 37·3 flowered in the case of the April flush, and 32·5 in the June flush in 1933, showing a slight increase in the number of flowering shoots in the April flush. Out of the shoots that flowered in 1933, a much smaller number made extension growth as compared with those that did not flower, showing an inverse correlation between growth and productivity during the same year. Again if the

flushes are compared between themselves, more shoots grew in the case of the April flush. In 1934 greater number of shoots flowered out of those which did not flower in the previous year. And conversely a smaller number of shoots flowered out of those which flowered in the previous year in the case of the April flush and none at all in the case of the June flush, thus showing a tendency of alternate bearing on the same shoots.

In 1934 also the April flush continued to be more important than the June flush with respect to productivity.

4. Periodicity of extension growth, 1935

(*a*) *Shoots of different flushes selected in 1935 and allowed to grow without imposing any other treatment on them*

The mean total extension growth in centimetres per shoot of different flushes is given in Table XI and shown graphically in Fig. 4. The calculated mean growth per shoot in percentages on the final length of the shoot is also given in this table.

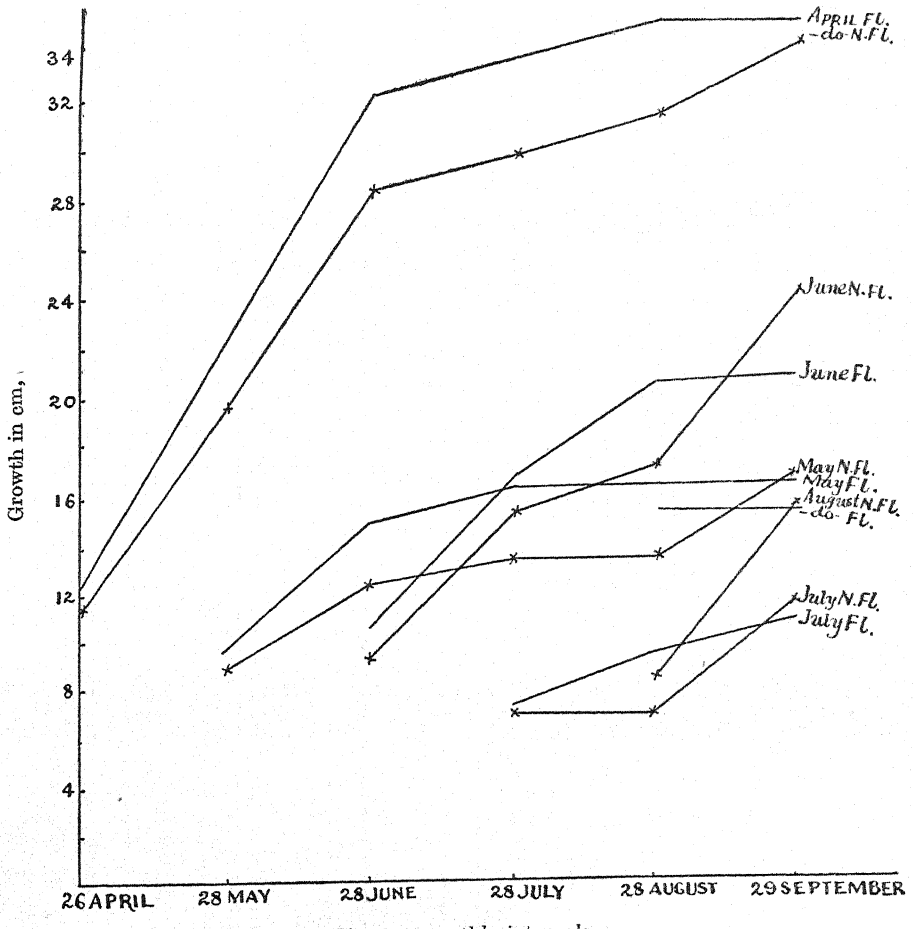

Fig. 4. Mean extension growth in 1935 of 'would-be flowering' and 'would-be non-flowering' shoots

April flush.—In shoots that flowered in the following year the rate of extension growth during May, June and July was more than the ' would-be non-flowering ' ones, after which for a month the latter shoots grew at a slightly more rapid rate than the former ones. During the last month of the growing season the ' would-be flowering ' shoots almost ceased growing, whereas the others continued growth at a rapid rate.

May flush.—The ' would-be flowering ' and ' would-be non-flowering ' shoots of this flush behaved in exactly the same way as those of the April flush. During the earlier part of the growing season, the growth rate of 'would-be flowering ' shoots was more than the ' would-be non-flowering' ones, whereas during the last month it was quite the reverse.

June flush.—The behaviour of shoots in both the categories was in conformity with those of other flushes already mentioned above.

July flush.—The ' would-be flowering ' shoots grew at a quicker rate than the ' would-be non-flowering ' ones till August, after which the growth in the former slowed down considerably, whereas the latter grew during the first month, stopped altogether for the next and started to grow at a rapid rate during the last month of the growing season.

August flush.—The ' would-be flowering ' shoots grew only during the month of August, and stopped extension growth altogether in September, whereas the non-flowering shoots went on growing till the end of the growing season.

From the above data on the shoots of all the flushes it is borne out clearly that the shoots that flowered in subsequent year made extension growth at rapid rate early in the season and stopped growing about a month earlier as compared to the shoots which remained barren in the following spring.

The calculated extension growth in percentages on the final length of the shoots in Table XI shows that invariably in all flushes the percentage of growth produced by the shoots that flowered in subsequent year was much more than the others throughout the early part and major portion of the growing season.

(*b*) *Deblossomed shoots*

The mean total extension growth, actual and calculated in percentages, of shoots that were deblossomed in early spring 1935 is given in Table XII. The mean total extension growth is shown graphically in Fig. 5.

In Fig. 5 it is quite interesting to note that extension growth in both the categories of shoots (i.e., those flowering or not flowering in the following year) did not take place during April and May. In June both the types of shoots started to grow but the ' would-be flowering ' ones evidently grew earlier and at a much quicker rate than the others. By the end of July the ' would-be flowering ' shoots had made slightly greater growth than the ' would be non-flowering ' ones. In the following two months the ' would-be flowering ' shoots (i.e. flowering in the subsequent spring 1936) made very little growth, while the others that remained barren in the subsequent spring made

tremendous growth during the month. Table XII shows that out of the total extension growth made by shoots of both categories the ' would-be flowering ' shoots made 97·4 per cent against only 54·5 per cent in the case of ' would-be non-flowering ' ones by the end of August.

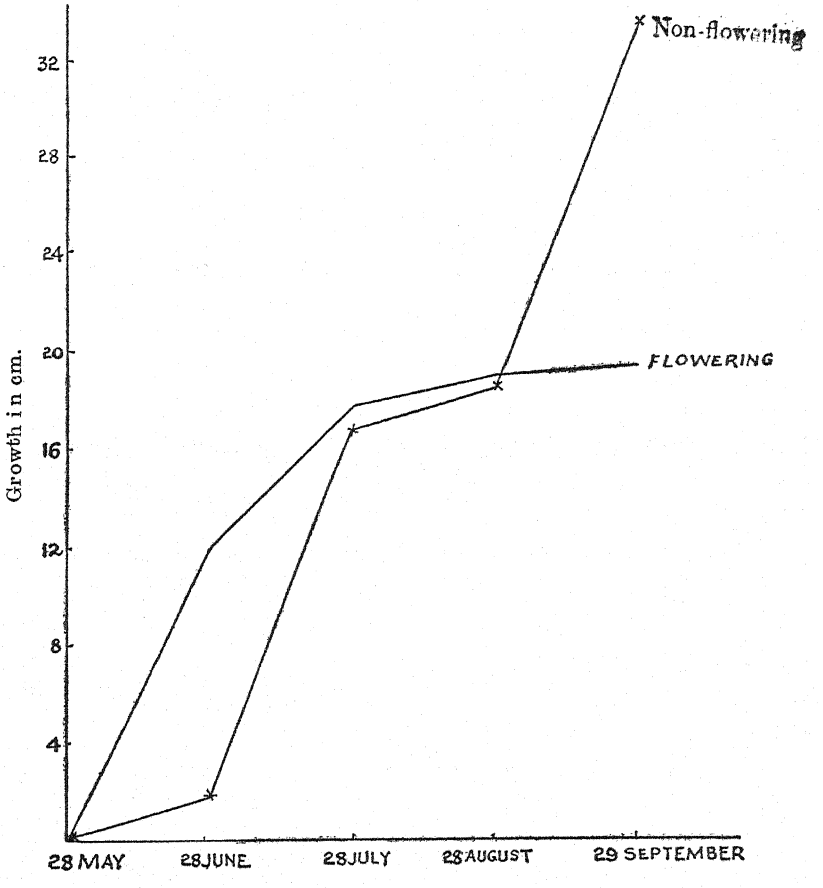

FIG. 5. Mean extension growth in 1935 of ' would-be flowering ' and ' would-be non-flowering ' shoots deblossomed in 1935

5. GROWTH PERFORMANCE AND FLOWERING RECORDS OF SHOOTS FROM 1935-38

(*a*) *Shoots of different flushes selected in 1935 and allowed to grow without imposing any other treatment on them*

1. Actual data regarding the growth and flowering records from 1935 to 1938 of different flushes selected in 1935 are presented in Table XIII.

Table XIII is self-explanatory excepting columns 6 and 7 which need explanation. In column 6, (*a*) shows the number of shoots growing out of flowering shoots in the previous column ; (*b*) shows the number of shoots growing out of the non-flowering shoots in the previous column ; and (*c*) shows the number of shoots growing out of shoots (in column 4) which did not grow in 1936. Column 7 shows the number of growths produced on the total number of growing shoots in the previous column. It is evident from the table that out of the shoots that flowered in spring, smaller number made extension growth in the subsequent year as compared to the non-flowering ones. Also the flowering shoots in the subsequent year fruited in far less number than the others.

Table XIV, which is prepared from Table XIII, clearly indicates this behaviour of the shoots.

TABLE XIV

Percentages of shoots growing in 1936 and bearing in 1937 out of flowering and non-flowering shoots under each flush in 1936

Name of flush	No. of shoots flowering and non-flowering in 1936		Percentage No. of shoots growing in 1936	Percentage No. of shoots flowering in 1937	Remarks
April	(*a*) Flowering	156	31·4	1·9	
	(*b*) Non-flowering	44	79·5	13·6	
May	(*a*) Flowering	83	4·8	0·0	Shoots largely in shade
	(*b*) Non-flowering	17	5·8	0·0	
June	(*a*) Flowering	90	17·7	1·1	
	(*b*) Non-flowering	10	90·0	10·0	
July	(*a*) Flowering	7	..	..	
	(*b*) Non-flowering	3	100·0	66·0	
August	(*a*) Flowering	5	..	..	
	(*b*) Non-flowering	5	60·0	20·0	

Out of the shoots in each flush that flowered in 1936 very few made extension growth during the same year, and also flowered in less number in the following year as compared to the non-flowering shoots, thus showing firstly the tendency of individual shoots to bear in alternate years and secondly that growth in the preceding year predetermines the fruitfulness in the subsequent year or in other words there is a direct relationship between number of shoots growing in the preceding year and the number of shoots flowering in the following year.

2. Calculated data in percentages on the original number of shoots.—In order to elucidate the above observations more clearly the data of Table XIII are calculated in percentages on the original number of shoots selected under each flush in 1935 and presented in Table XV.

The table shows firstly that out of the shoots of each flush of 1935 the percentage of flowering shoots was very much higher in 1936 than in 1937 and it again went up in 1938. Secondly the relation of number of shoots growing in the preceding year and the number of shoots flowering in the following year was again established by the data in Table XV. Thirdly there was an antagonism between the number of shoots growing and number of shoots bearing during the same season, i.e. vegetative growth was at the expense of fruitfulness and *vice versa*.

If the mean of all the flushes is taken as shown in Table XV, it would show the behaviour of the tree as a whole as the representative shoots from each flush were selected on the tree. The behaviour of the tree as a whole with regard to growth and productivity is the same as mentioned above for each flush.

The relative importance of different flushes with respect to productivity is also shown in column 3 of Table XV, where it is evident that three flushes initiated earlier were more important than the other two initiated later.

(*b*) *Deblossomed shoots*

It has been clearly demonstrated that (*a*) shoots bearing fruits seldom make extension growth during the season and seldom bear fruit next year and (*b*) shoots making extension growth largely bear crop next season provided growth ceases fairly early in the season. It would be interesting to note how the shoots, actually in flowering would behave with regard to extension growth during the season and fruiting during the next season in case they are deblossomed.

Table XVI shows that shoots that were deblossomed in 1935 grew again in the same year and out of the 27 shoots no less than 20 flowered in the following year. The shoots that flowered in 1936 did not grow again during the same year, while of the others which did not flower in 1936 all excepting one grew. In 1937 no shoot flowered out of those flowering in 1936 and two flowered out of those that did not flower in the previous year.

The data from Table XVI is calculated in percentages on the original number of shoots deblossomed in 1935 and presented in Table XVII which shows that: firstly deblossoming the shoots induced them to grow again during the same year and to flower in the subsequent years; secondly the percentage of flowering shoots in 1936 was more than in 1937, and in 1938 again the percentage of flowering shoots went high, thus showing the tendency of individual shoots to bear in alternate years; thirdly there was a positive correlation between growth in the preceding year and productivity in the following year; and fourthly there was an antagonism between the number of

flowering and growing shoots during the same year and all this is in conformity with the results obtained in the case of normal shoots not deblossomed.

6. Periodicity of extension growth, 1937

The mean total extension growth in centimetres and calculated growth on percentage basis per shoot of various flushes selected in 1937 are given in Table XVIII and shown graphically in Fig. 6.

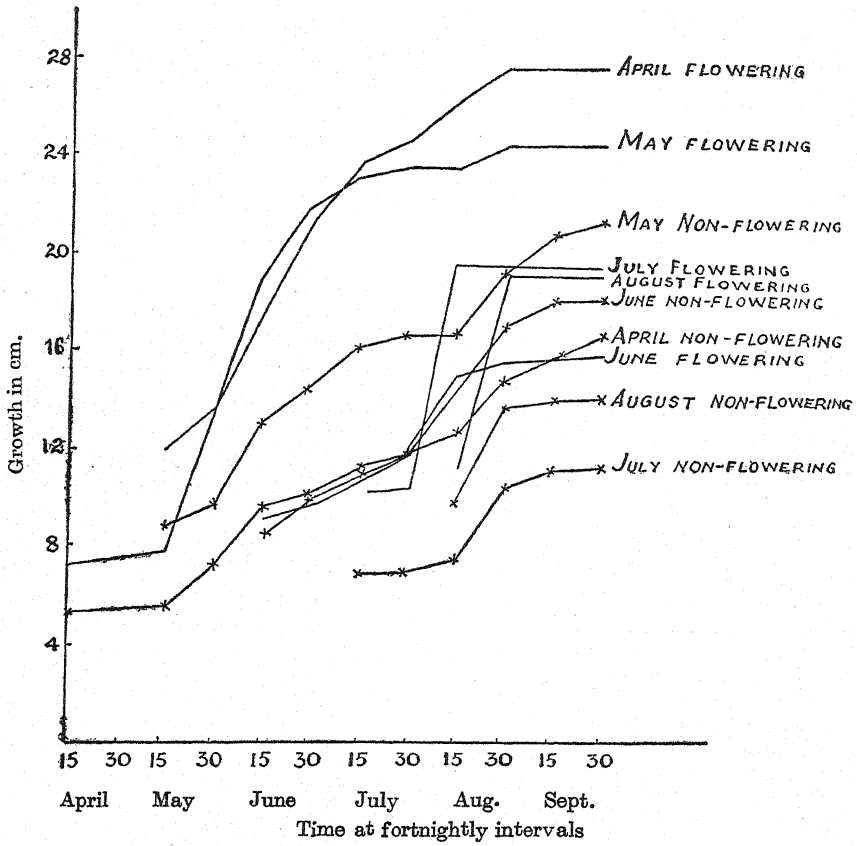

Fig. 6. Mean extension growth in 1937 of 'would-be flowering' and 'would-be non-flowering' shoots

April flush.—The rate of growth of 'would-be flowering' and 'would-be non-flowering' shoots from April 15th to May 15th was the same after which period till 15th July the growth was progressing at a rapid rate in the case of former shoots, whereas in the latter ones the growth during this period was slow. From 15th July to 30th August the growth rate was practically the same in both the categories of shoots after which the 'would-be flowering' shoots stopped altogether while the others went on growing till the end of September.

May flush.—From 15th May to 30th June the growth rate in case of 'would be flowering' shoots was slightly more than the 'would-be non-flowering' ones after which till 15th August both the types of shoots were growing at the same rate. After 15th August the 'would-be non-flowering' shoots grew at a rapid rate till the end of September while 'would-be flowering' shoots stopped growing as early as the end of August.

June flush.—The growth of both the categories of shoots was at the same rate till 30th July after which for a fortnight the 'would-be flowering' shoots were growing comparatively rapidly. From 15th to 30th August the 'would-be non-flowering' shoots grew at a rapid rate, whereas the 'would-be flowering' ones during this period slowed down considerably and ceased growth by 30th August. The 'would-be non-flowering' shoots went on growing till the middle of September.

July flush.—Both the types of shoots stopped growing from 15th to 30th July after which for a fortnight the 'would-be flowering' shoots grew at a more rapid rate than the 'would-be non-flowering' ones. On 15th August the growth of 'would-be flowering' shoots ceased, whereas the 'would-be non-flowering' ones went on growing till 15th September, after which the growth slowed down and finally stopped at the end of September.

August flush.—The growth of 'would-be flowering' shoots was more than the 'would-be non-flowering' ones from 15th to 30th August. After this the former ceased growing altogether, whereas in the latter the growth slowed down.

From the above it is evident that the 'would-be flowering' shoots (i.e. those that flowered in the subsequent spring) in every flush produced more growth earlier in the growing season and stopped earlier, whereas the 'would-be non-flowering' shoots produced more growth during the latter part of the growing season and continued to grow till late in the season. All the shoots that flowered in subsequent year stopped growth by the end of August, while the others continued to grow till the end of September.

Table XVIII, besides showing the actual extension growth at fortnightly intervals, also shows the growth in terms of percentages which elucidates even more clearly the points referred to above.

7. Relative importance of various flushes with respect to productivity

From column 2 of Table XVIII the percentage number of shoots that flowered in each flush is calculated and set out below.

Name of flush	April	May	June	July	August
Percentage number of shoots flowering . .	36·7	29·4	19·8	11·0	7·0

The table shows that the shoots initiated earlier in the growing season came into flower in greater number than those which started to grow late, emphasizing that the earlier flushes are more important from the point of view of productivity.

IV. Discussion

The seasonal elongation of shoots, as observed over a number of years on a considerable number of shoots growing on several or individual trees, revealed that the flowering and non-flowering shoots (i.e. the shoots that in the subsequent spring were to flower or remain barren) exhibited in the previous season a marked difference in their growth periodicity. The flowering shoots in the preceding year grew at a much rapid rate and put on relatively more growth during the early part of the growing season as well as ceased growing about a month earlier than those which did not flower. The non-flowering shoots during the preceding year grew at a lower rate in the beginning and went on growing till late in the season. The association of more amount of growth during the early part of the growing season and cessation of extension growth earlier to fruit bud formation is conclusively established in shoots of the Langra mango under Lyallpur conditions. The reason for this behaviour of the shoots can be sought in their period of leaf activity and maturation of their wood. Khan [1938], working on apples, has shown the similarity of curves for total leaf area and total shoot growth. This shows that periodicity of extension growth and seasonal development of leaf area are associated, and it is reasonable to believe that the same relation also holds good in the case of the fruit under study. Consequently leaf area in the case of shoots developing and stopping earlier remained active over a greater period in the growing season as compared to other shoots, and was thus responsible for fruit bud differentiation. Hartless [1913] has mentioned that hard, ripe wood in mango is very essential for fruiting, and flowering shoots of mango are usually produced in late spring and summer. He mentions that he saw a very good crop of mango following a monsoon that closed completely fairly early in September and that a prolonged monsoon was inimical to a good mango season the following year. In fact research on this phase of the problem on apples and other fruits has also shown that any treatment, whether rootstock, pruning, ringing or any cultural practice that induce this type of growth are conducive to fruit bud formation. It has been shown by Swarbrick [1927] that under certain conditions ringing is a sure way of bringing about flower bud formation in over-vegetative trees. Lees [1926] has pointed out that in England dry summers are more conducive to flower bud formation than the wet ones. Again certain vegetative rootstocks have also been shown to bring about precocity in the scions worked upon them [Hatton, 1920, 1925]. It is well known also that fruit trees under sod or some form of cover crops are more prolific of fruit bud formation than those under clean cultivation provided nitrogen is not a limiting factor. All the above conditions have a common influence on tree growth in that they retard the vegetative growth and induce its cessation relatively earlier in the season.

The second point to be discussed is the growth performance and flowering records of shoots from year to year. It was found in this connection that in mature trees there was an antagonism between productivity and growth on the same shoots during the same growing season. The shoots bearing fruit either did not grow or grew in very small number during the same growing season, whereas non-fruiting shoots grew in greater number. Secondly the shoots that flowered in one season either did not flower at all or flowered in very small number in the following year, showing a tendency to bear biennially. Thirdly only those shoots flowered which grew and stopped growth earlier in the preceding year. The shoots that did not grow did not flower in the following year, showing that flowering in Langra mangoes took place only on one year old growths and consequently there was a direct relationship between the number of shoots growing in a season and the number of shoots flowering in the subsequent year, provided of course the number of growing shoots produced desirable type of extension growth required for fruit bud formation. From the above information it is evident that lack of fruiting on the shoots of Langra mangoes in any particular year is brought about through lack or absence of the desirable type of extension growth in the preceding year. This, combined with the antagonism of fruiting to vegetative growth, brings about biennial bearing. This would explain partly why young mango trees comparatively bear regularly every year, since along with fruiting, adequate amount of growth is also being produced. If by some means a balance between growth and flowering is maintained every year, it should be possible to secure a reasonable crop year after year. It would be desirable to mention here how throughout the province there is very little or no mango crop in one year and plenty in another which should not happen in the normal course on account of the fact that all mango plantations are not of the same age. This phenomenon of biennial bearing in mango throughout the mango-growing areas is brought about by external factors, like frost or pests and diseases, which annihilate either the potential fruit buds or crop throughout and disturb the enquilibrium of the trees. During the year if the crop is damaged all the energy of the trees is expended in the production of vegetative growth and during the next year if there is no external calamity the trees blossom profusely and fruit in abundance with no vegetative growth to provide for the crop during the next year. Once this happens the trees tend to bear biennially unless of course this habit is again changed by the external factors mentioned above. It stands to reason that in a year of heavy fruiting, any cultural treatment tending to encourage extension growth of shoots should bring about fruiting in the subsequent year also and thus break the biennial bearing habit of the tree.

It has been shown that the shoots that ceased growth earlier became productive irrespective of their age, though, of course the number of such shoots was greater in flushes initiated earlier. April, May and June flushes were found to be more important than July and August flushes from the point of view of fruitfulness. Excepting one year the order of importance even in the three earlier flushes was according to the time these were initiated,

i.e. April flush was more important than the June flush and June flush in turn was more important than July flush. If comparison is made between July and August flushes the former was more important than the latter. This information throws some light on the importance of early growth initiation. And to get a reasonable quantity of crop in mangoes under Lyallpur conditions it is very essential that the growth initiation should take place fairly early in the preceding season. In the light of this it seems desirable to modify the cultural practices in a way so as to bring about maximum growth fairly early in the season.

Deblossoming the shoots early in the spring resulted in inducing the deblossomed shoots to grow in the same season and behave like other shoots not bearing fruit, and about 70 per cent of these shoots flowered and fruited again in the subsequent year. This response of mango to deblossoming seems to be of vital importance in regulating the crop every year, since by adopting this practice it is shown to be possible to induce vegetative growth in the tree during the same year and bring about fruiting in the following year. Overbearing during one year brings about biennial bearing in the mature mango trees, and by deblossoming this habit can be averted and the trees brought into bearing annually. These results are supported by the evidences of other workers on other fruits. Aldrich [1931] has shown that thinned trees set more blossom in the subsequent year than the unthinned ones. Aldrich and Fletcher [1932] have proved that heavy fruit thinning within six weeks of blossoming dates is apparently effective in increasing the fruit bud formation for the following year. McCormick and others [1933] have pointed out that pre-thinning (a term used to denote the procedure of removing blossom clusters at or about blooming time) would correct biennial bearing in apples. Potter [1936] has shown that biennial bearing is due to an early drain on the tree caused by the many fruit developing prior to June drop and early thinning may be expected to increase the amount of bloom in the succeeding year. He has shown that on an average 25 per cent of the spurs on the trees thinned earlier in the season produced blossom again in the following year.

V. Summary and conclusion

1. The paper deals with the investigations carried out at Lyallpur from 1932 to 1938 on the relation of growth to bearing in mangoes.

2. Six uniform grafted Langra mango trees of the same age growing on seedling rootstocks and under uniform cultural and other treatments were included in the investigation in 1932 and kept under observation up to the spring of 1934. In 1935 only one tree was included in the project to eliminate the element of uncertainty with respect to the ' build up ' of the tree and this tree was kept under observation till the spring of 1938. In 1937 another tree was included in the experiment and kept under study till 1938.

3. From the above trees, flushes initiated during different months of the growing seasons on the last year's growths were selected and designated according to the month in which they started to grow.

4. Shoots of different flushes were labelled and seasonal elongation of each shoot was recorded after suitable intervals of one week, two weeks and a month in different years till the extension growth stopped altogether. In the following year the behaviour of each individual shoot was noted with respect to flowering or otherwise. They were grouped into flowering and non-flowering shoots and the means of seasonal elongation of growth of these shoots in the previous year computed separately for both types of shoots. In the text of this paper, while discussing the behaviour of these shoots in regard to their growth per odicity prior to flowering, the terms ' would-be flowering ' shoots and ' would-be non-flowering ' shoots have been used for these shoots.

5. In addition to the study of seasonal elongation of shoots and its bearing on fruiting in the following year, observations on these shoots were continued for a couple of years in regard to their growth and flowering performance.

6. Flowering shoots were deblossomed and their behaviour in regard to seasonal elongation during the year of deblossoming as well as flowering or otherwise in the following year was studied and this was continued for four years.

7. The data was examined statistically whenever the material warranted such examination.

8. ' Would-be flowering' and ' would-be non-flowering ' shoots exhibited a marked difference in their growth periodicity during the year preceding flowering. Shoots that flowered in the subsequent year made most of the extension growth early in the season and ceased growing about a month earlier as compared to the shoots that did not flower in the following spring. This was consistently observed over a period of four years on about 1,300 shoots of April, May, June, July and August flushes.

9. An antagonism between productivity and growth on the same shoots during the growing season was established, i.e. fruiting and vegetative growth were always at the expense of each other.

10. There was a direct relationship between the number of shoots growing in one season and the number of shoots flowering in the subsequent spring.

11. The shoots that flowered in one season either did not flower or flowered in very small number in the following year, thus indicating a tendency of alternate bearing on individual shoots. The causes of ' on ' or ' off ' years in mangoes throughout the mango-growing tracts are also discussed.

12. The fruit-bud initiation took place only on one season old shoots.

13. The flushes initiated early were more important than those initiated late in the season with respect to productivity in the following year.

14. Deblossoming early in the season resulted in inducing the shoots to grow in the same season and bear in the next. They behaved exactly in the same way as shoots that did not flower.

VI. Acknowledgment

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Appendix

TABLE I

Mean total extension growth in 1932 of 'would-be flowering' and 'would-be non-flowering' shoots of April flush

No. of shoots under observation	Extension growth in 1932 in centimetres												Behaviour in 1933
	23/4	29/4	6/5	11/5	21/5	16/6	23/6	29/6	5/7	11/7	17/7	21/7	
50	9·5	15·0	15·8	16·8	19·3	20·4	22·5	24·4	25·7	25·7	25·7	25·7	Flowering
84	4·3	8·9	10·1	13·7	13·7	16·0	19·2	22·1	22·5	23·0	23·5	23·6	Non-flowering

N.B.—Altogether 134 shoots had been selected in April 1932. Out of these 50 shoots flowered in the following spring (i.e., 1933) and 84 did not flower. Their behaviour with respect to their growth during the year preceding flowering is given above.

TABLE II

Data of Table I is represented on percentage basis (S. E. of the mean 2·35, sig. diff. 7·46)

No. of shoots under observation	Percentage extension growth in 1932												Behaviour in 1933
	23/4	29/4	6/5	11/5	21/5	16/6	23/6	29/6	5/7	11/7	17/7	21/7	
50	36·9	58·4	61·5	65·4	75·1	79·4	87·6	94·9	100·0	100·0	100·0	100·0	Flowering
84	18·2	37·7	42·8	58·0	58·0	67·7	81·3	93·6	95·3	97·4	99·5	100·0	Non-flowering

TABLE III

Mean total extension growth in 1932 of 'would-be flowering' and 'would-be non-flowering' shoots of June flush

No. of shoots under observation	Extension growth in 1932 in centimetres								Behaviour in 1933
	15/6	21/6	27/6	3/7	9/7	15/7	21/7	27/7	
39	5·8	7·1	7·5	8·3	9·2	9·9	9·9	9·9	Flowering
81	5·5	6·5	6·7	7·5	8·1	8·5	10·6	10·7	Non-flowering

NOTE.—Altogether 120 shoots had been selected in June 1932. Out of these 39 shoots flowered in the following spring (1933) and 81 did not flower. Their behaviour with respect to their growth during the year preceding flowering is given above.

TABLE IV

Data of Table III represented on percentage basis (S. E. of the mean 2·12, sig. diff. 6·64)

No. of shoots under observation	Percentage extension growth in 1932								Behaviour in 1933
	15/6	21/6	27/6	3/7	9/7	15/7	21/7	27/7	
39	58·6	71·7	75·8	83·9	92·9	100·0	100·0	100·0	Flowering
81	51·4	60·7	62·6	70·0	75·7	79·4	99·0	100·0	Non-flowering

TABLE V

Mean total extension growth in 1933 of 'would-be flowering' and 'would-be non-flowering' shoots of April and June flush shoots non-bearing in 1933

Name of flush selected in 1932	No. of shoots non-bearing in 1933	No. of shoots that made extension growth in 1933	Behaviour of shoots in 1934	Extension growth in 1933 in cm.				
				20/4	20/5	20/6	20/7	22/8
April	84 (*vide* Table I)	56	Flowering 40	6·4	8·8	21·5	25·4	25·4
			Non-flowering 16	3·1	7·0	12·1	16·0	18·5
June	81 (*vide* Table III)	35	Flowering 22	1·9	3·5	11·6	15·7	15·7
			Non-flowering 13	0·3	1·5	6·9	12·9	15·6

TABLE VI

Data of Table V represented on percentage basis (S. E. of the mean 1·35, sig. diff. 4·25)

Name of flush selected in 1932	No. of shoots non-bearing in 1933	No. of shoots that made extension growth in 1933	Behaviour of shoots in 1934	Percentge extension growth in 1933				
				20/4	20/5	20/6	20/7	22/8
April	84 (*vide* Table I)	56	Flowering 40	25·2	34·7	84·7	100·0	100·0
			Non-flowering 16	16·7	37·8	65·4	86·5	100·0
June	81 (*vide* Table III)	35	Flowering 22	12·1	22·3	74·0	100·0	100·0
			Non-flowering 13	1·9	9·6	44·2	82·7	100·0

TABLE VII

Mean total extension growth in 1933 of 'would-be flowering' and 'would-be non-flowering' shoots of April and June flush shoots bearing in 1933

Name of flush selected in 1932	No. of shoots bearing in 1933	No. of shoots that made extension growth in 1933	Behaviour of shoots in 1934	Extension growth in 1933 in cm.				
				20/4	20/5	20/6	20/7	22/8
April	50 (*vide* Table I)	15	Flowering 2	..	..	22·3	30·3	30·3
			Non-flowering 13	2·8	4·9	10·6	12·9	19·0
June	39	2	Flowering ..	..	..	..	..	..
	(*vide* Table III)		Non-flowering 2	..	2·0	2·7	7·0	14·0

TABLE VIII

Data of Table VII represented on percentage basis

Name of flush selected in 1932	No. of shoots bearing in 1933	No. of shoots that grew in 1933	Behaviour of shoots in 1934	Percentage extension growth in 1933				
				20/4	20/5	20/6	20/7	22/8
April	50	15	Flowering 2	..	..	73·6	100·0	100·0
			Non-flowering 13	14·7	25·7	55·8	67·8	100·0
June	39	2	Flowering ..	..	..	..	..	..
			Non-flowering 2	..	14·3	19·3	50·0	100·0

TABLE IX

Growth performance and flowering record from 1932 to 1934 of April and June flush shoots selected in 1932

Name of flush	No. of shoots selected and growing in 1932	No. of shoots flowering in spring 1933		No. of shoots growing in 1933	No. of shoots flowering in spring 1934	
April	134	Flowering	50	15	Flowering	2
					Non-flowering	13
		Non-flowering	84	56	Flowering	40
					Non-flowering	16
June	120	Flowering	39	2	Flowering	*Nil*
					Non-flowering	2
		Non-flowering	81	35	Flowering	22
					Non-flowering	13

TABLE X

Growth performance and flowering record from 1932 to 1934 of April and June flush shoots (selected in 1932) expressed in percentages

Name of flush	Percentage of growing shoots selected in 1932	Percentage of flowering and non-flowering shoots in 1933 (*a*) Flowering (*b*) Non-flowering	Percentage of shoots growing in 1933 out of the shoots in the previous column	Percentage flowering shoots in 1934 out of the shoots in column 3
April	100·0	(*a*) 37·3	30·0	4·0
		(*b*) 62·7	66·0	47·6
June	100·0	(*a*) 32·5	5·1	0·0
		(*b*) 67·5	43·2	27·1

TABLE XI

Mean total extension growth (in cm.) and calculated growth in percentages in 1935 of 'would-be flowering' and 'would-be non-flowering' shoots of various flushes

Name of flush	No. of shoots under observation in 1935	Extension growth both actual and calculated in 1935						No. of shoots 1936 (*a*) Flowering (*b*) Non-flowering	Actual or calculated growth
		April	May	June	July	August	September		
April	200	12·3	22·2	32·4	33·9	35·5	35·6	(*a*) 156	Actual
		%34·5	62·4	91·0	95·2	99·7	100·0		Calculated
		11·4	19·7	28·6	30·2	31·8	34·8	(*b*) 44	Actual
		%32·7	56·6	82·2	86·8	91·4	100·0		Calculated
May	100	..	9·7	14·9	16·4	16·6	16·7	(*a*) 83	Actual
		..	%58·1	89·2	98·2	99·4	100·0		Calculated
		..	9·0	12·4	13·5	13·5	17·0	(*b*) 17	Actual
		..	%52·0	72·9	79·4	79·4	100·0		Calculated
June	100	..	..	10·6	16·8	20·7	21·0	(*a*) 90	Actual
				%50·5	80·0	98·5	100·0		Calculated
		..	..	9·3	15·4	17·3	24·5	(*b*) 10	Actual
		..	..	%37·9	62·8	70·6	100·0		Calculated
July	10	..	..	..	7·5	9·7	11·1	(*a*) 7	Actual
		..	..	..	%67·5	87·4	100·0		Calculated

		..	..	..	7·2	7·2	11·8	} (b) 3		Actual
		..	..	..	%61·0	61·0	100·0			Calculated
August	10	..	..	..	..	15·5	15·5	} (a) 5		Actual
		..	..	..	..	%100·0	100·0			Calculated
		..	..	..	..	8·7	15·7	} (b) 5		Actual
		..	..	..	..	%55·4	100·0			Calculated

TABLE XII

Mean total extension growth (in cm.) and calculated growth in percentages in 1935 of 'would-be flowering' and 'would-be non-flowering' shoots deblossomed in 1935

Total number of shoots deblossomed in 1935	No. of shoots flowering in 1936		Extension growth in 1935				Actual or calculated growth
			June 28	July 28	August 28	September 29	
27	Flowering	20	11·8	17·7	19·0	19·5	Actual
			%60·5	90·8	97·4	100·0	Calculated
	Non-flowering	7	1·7	16·7	18·6	33·6	Actual
			%5·1	49·7	55·4	100·0	Calculated

TABLE XIII

Growth performance and flowering records from 1935 to 1938 of shoots of various flushes selected in 1935

Name of flush	No. of shoots selected and growing in 1935	No. of shoots in 1936 (*a*) flowering (*b*) non-flowering	No. of shoots in 1936 (*c*) growing (*d*) non-growing	No. of shoots in 1937 (*a*) flowering (*b*) non-flowering	No. of shoots growing in 1937	No. of growths produced on shoots in col. 6	No. of shoots flowering in 1938 out of the shoots in column 7	Remarks
April	200	(*a*) 156	(*c*) 49 (*d*) 107	(*a*) 3 (*b*) 46	(*a*) — (*b*) 36 *(*e*) 46	107	186	125	*(*e*) in col. 6 shows the No. of shoots growing out of shoots in col. 4 which did not grow in 1936
		(*b*) 44	(*c*) 35 (*d*) 9	(*a*) 6 (*b*) 29	(*a*) — (*b*) 21 (*e*) 4			
May	100	(*a*) 83	(*c*) 4 (*d*) 79	(*a*) — (*b*) 4	(*a*) — (*b*) 3 (*e*) 9	15	21	10	Most of the shoots were in shade and did not grow probably due to this reason.
		(*b*) 17	(*c*) 1 (*d*) 16	(*a*) — (*b*) 1	(*a*) — (*b*) 1 (*e*) 2			
June	100	(*a*) 90	(*c*) 16 (*d*) 74	(*a*) 1 (*b*) 15	(*a*) — (*b*) 4 (*e*) 16	24	46	15	
		(*b*) 10	(*c*) 9 (*d*) 1	(*a*) 1 (*b*) 8	(*a*) — (*b*) 4 (*e*) —			

July	10	(*a*) 7 (*b*) 3	(*c*) — (*d*) 7 (*c*) 3 (*d*) —	(*a*) — (*b*) — (*a*) 2 (*b*) 1	(*a*) — (*b*) — (*e*) 2 (*a*) 1 (*b*) 1 (*e*) —	4	5	4
August	10	(*a*) 5 (*b*) 5	(*c*) — (*d*) 5 (*c*) 3 (*d*) 2	(*a*) — (*b*) — (*a*) 1 (*b*) 2	(*a*) — (*b*)— (*e*) — (*a*) — (*b*) 2 (*e*) 1	3	5	3

TABLE XV

Data of Table XIII regarding number of flowering and growing shoots represented on percentage basis

Name of flush	Percentage of growing shoots selected in 1935	Percentage of shoots flowering in 1936	Percentage of shoots growing in 1936	Percentage of flowering shoots in 1937	Percentage of growing shoots in 1937	Percentage of flowering shoots in 1938	Remarks
April	100·0	*78·0*	42·0	*4·5*	93·0	*62·5*	
May	100·0	*83·0*	5·0	..	21·0	*10·0*	
June	100·0	*90·0*	25·0	*2·0*	46·0	*15·0*	
July	100·0	*70·0*	30·0	*20·0*	50·0	*40·0*	
Angust	100·0	*50·0*	30·0	*10·0*	50·0	*30·0*	
Total	500·0	371·0	132·0	36·5	260·0	157·5	
Mean	100·0	*74·2*	26·4	*7·3*	52·0	*31·5*	

TABLE XVI

Growth performance and flowering records from 1935 to 1938 of shoots deblossomed in the spring of 1935

No. of shoots deblossomed in 1935	No. of shoots growing in 1935	No. of shoots (*a*) flowering (*b*) non-flowering in 1936	No. of shoots growing in 1936	No. of growths on shoots in the previous column	No. of shoots flowering in 1937	No. of shoots growing in 1937	No. of growths on the shoots in the previous column	No. of shoots flowering in 1938
27	27	(*a*) *20*	..	..	..	7	19	7
		(*b*) 7	6	14	*2*	2		

TABLE XVII

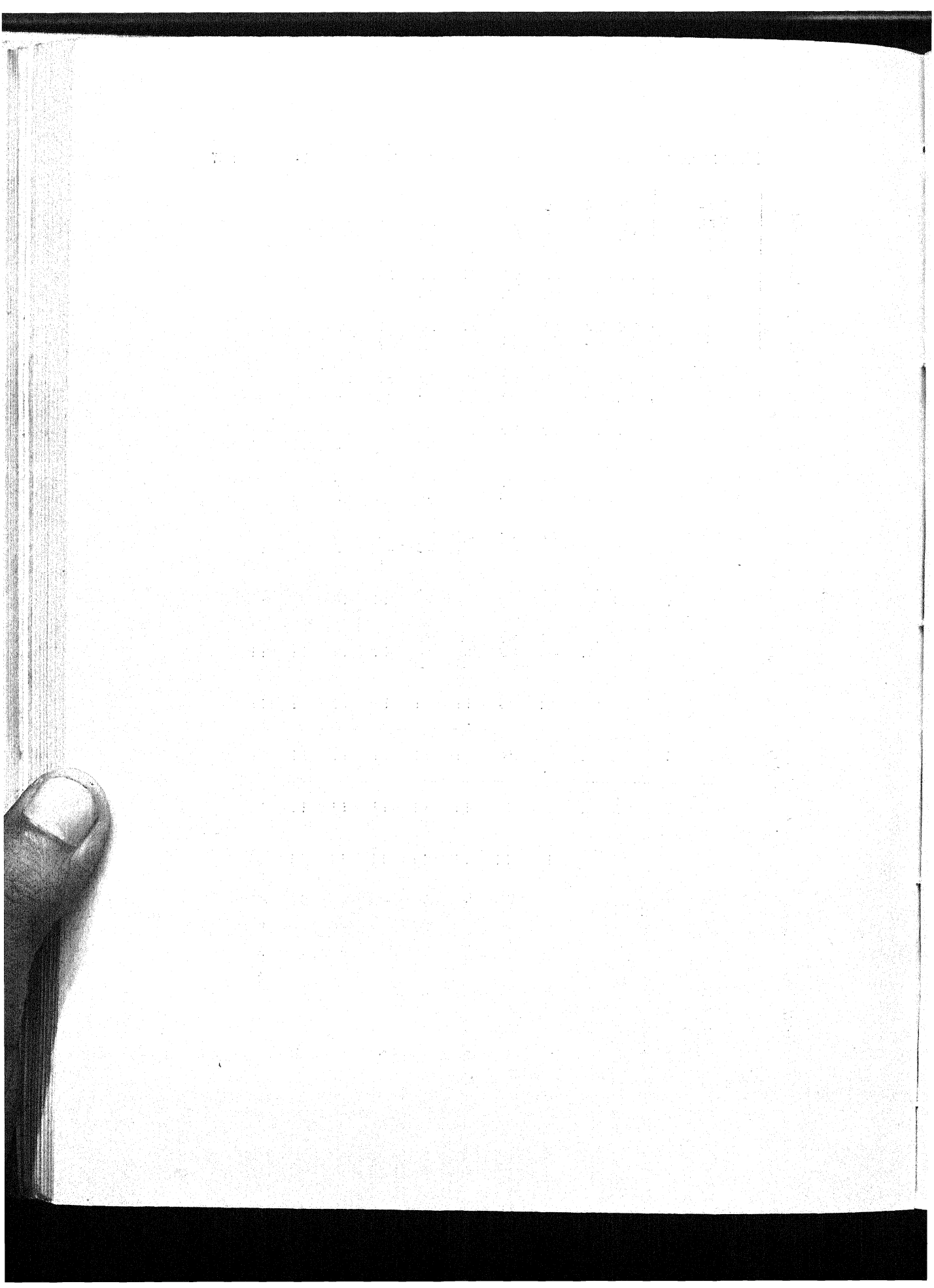
The data of Table XVI is represented on percentage basis on the original number of shoots

Percentage of shoots deblossomed in 1935	Percentage of shoots growing in 1935	Percentage of shoots flowering in 1936	Percentage of shoots growing in 1936	Percentage of shoots flowering in 1937	Percentage of shoots growing in 1937	Percentage of shoots flowering in 1938
100·0	100·0	*74·1*	51·8	*7·8*	70·4	*26·0*

TABLE XVIII

Mean total extension growth (in cm.) and calculated growth in percentages in 1937 of 'would-be flowering' and 'would-be non-flowering' shoots of various flushes

Name of flush	Number of shoots under observations in 1938		Mean shoot growth in cm. during 1937												Actual or calculated growth
			April		May		June		July		August		September		
			15	30	15	30	15	30	15	30	15	30	15	30	
April	Flowering	33	7·3	7·5	7·7	13·3	17·6	21·3	23·7	24·6	26·2	27·5	27·5	27·5	Actual
			%26·6	27·3	28·0	48·4	64·0	77·5	86·2	89·5	95·3	100·0	100·0	100·0	Calculated
	Non-flowering	57	5·3	5·5	5·6	7·2	9·6	10·2	11·3	11·8	12·7	14·9	15·8	16·6	Actual
			%31·9	33·1	33·7	43·4	57·8	61·4	68·8	71·0	76·5	89·7	95·1	100·0	Calculated
May	Flowering	28	...	...	11·9	13·6	18·9	21·8	23·1	23·5	23·5	24·4	24·4	24·4	Actual
			...	...	%48·8	55·7	77·5	88·5	94·6	96·4	96·4	100·0	100·0	100·0	Calculated
	Non-flowering	67	...	...	8·8	9·7	13·1	14·6	16·1	16·6	16·6	19·2	20·7	21·3	Actual
			...	...	%41·3	45·5	61·5	68·5	75·5	77·9	77·9	90·1	97·2	100·0	Calculated
June	Flowering	19	...	...	...	...	9·1	9·9	11·2	11·8	15·0	15·3	15·3	15·3	Actual
			...	...	...	...	%59·5	64·7	73·2	77·1	98·0	100·0	100·0	100·0	Calculated
	Non-flowering	77	...	...	...	...	8·5	10·1	11·5	11·5	12·6	16·5	18·0	18·0	Actual
			...	...	...	...	%47·2	56·1	63·8	63·8	70·0	91·7	100·0	100·0	Calculated
July	Flowering	11	...	...	...	...	...	...	10·6	10·6	19·5	19·5	19·5	19·5	Actual
			...	...	...	...	...	...	%54·4	54·4	100·0	100·0	100·0	100·0	Calculated
	Non-flowering	89	...	...	...	...	...	...	7·0	7·0	7·6	10·4	11·2	11·3	Actual
			...	...	...	...	...	...	%61·9	61·9	67·2	92·0	99·1	100·0	Calculated
August	Flowering	7	...	...	...	...	...	...	...	...	11·2	19·0	19·0	19·0	Actual
			...	...	...	...	...	...	...	...	%58·9	100·0	100·0	100·0	Calculated
	Non-flowering	93	...	...	...	...	...	...	...	...	9·9	13·8	13·9	13·9	Actual
			...	...	...	...	...	...	...	...	%71·2	99·2	100·0	100·0	Calculated



NOTES

Notice No. 3 of 1939

THE following Plant quarantine regulations and import restrictions have been received in the Imperial Council of Agricultural Research. Those interested are advised to apply to the Secretary, Imperial Council of Agricultural Research, New Delhi, for loan.

I. LIST OF UNITED STATES DEPARTMENT OF AGRICULTURE, BUREAU OF ENTOMOLOGY AND PLANT QUARANTINE, SERVICE AND REGULATORY ANNOUNCEMENTS

1. Consolidated Service and Regulatory announcements, January-March 1939

2. Quarantine and other official announcements—

(*i*) Japanese Beetle Quarantine (No. 48)—Revision of regulations.

(*ii*) Pink Bollworm Quarantine (No. 52)—Revision of quarantine and regulations.

3. Summaries of plant quarantine import restrictions—

(*i*) *Union of South Africa.*—Restrictions on the importation of hay and straw as packing for merchandise.

(*ii*) *Eire* (*Irish Free State*).—Chrysanthemum Midge Order amended —importation of chrysanthemum plants prohibited.

(*iii*) *British Colony of Malta.*—Potatoes may be imported from Alexandria, Egypt.

(*iv*) *Iran* (*Persia*).—Disinfection certificate required for flower bulbs and ornamentals.

(*v*) *Commonwealth of Australia.*—Restrictions on the importation of vegetables—amendment of regulation 21 of the Regulations effective September 19, 1935.

(*vi*) *Commonwealth of Australia.*—Stone fruit trees from United States—Each State regarded as a country.

(*vii*) *Colony of St. Lucia* (*British West Indies*).—Importation of banana plants prohibited.

(*viii*) *Mandated Australian Territory of New Guinea.*—Digest of plant quarantine import restrictions.

(*ix*) *Republic of Finland.*—Precautions against foot-and-mouth disease.

(*x*) *British Colony and Protectorate of Sierra Leone.*—Revision of the digest of the plant quarantine import restrictions.

4. List of intercepted plant pests, 1937

II. OTHER ANNOUNCEMENTS

1. Nyasaland Protectorate—

(*i*) Proclamation No. 4 of 1939—import restrictions of certain plants, plant portions and plant products grown or produced in the territory administered by Companhia de Mozambique.

(*ii*) Proclamation No. 8 of 1939—import restrictions of certain plants or portions of plants from certain districts in the Cape Province of the Union of South Africa.

2. Colony of St. Lucia.—Proclamation—prohibition of importation of oil sands from Trinidad.

NOTIFICATION No. F. 7-6/39, DATED THE 15TH JUNE 1939, ISSUED BY THE GOVERNMENT OF INDIA, IN THE DEPARTMENT OF EDUCATION, HEALTH AND LANDS

THE following Order issued by the Ministry of Agriculture and Fisheries, London, called 'The Importation of Plants Order of 1939', is published for general information :—

STATUTORY RULES AND ORDERS

1939, No. 532

DESTRUCTIVE INSECT AND PEST, ENGLAND

THE IMPORTATION OF PLANTS ORDER OF 1939, DATED MAY 16, 1939 (D.I.P.-605)

The Minister of Agriculture and Fisheries by virtue and in exercise of the powers vested in him under the Destructive Insects and Pests Acts, 1877 to 1927[a], and of every other power enabling him in this behalf, orders as follows :—

Definitions

1. In this Order, unless the context otherwise requires :—

'Importer' includes any person who, whether as owner, consignor or consignee, agent or broker, is in possession of or in anywise entitled to the custody or control of the article.

'Inspector' means an Inspector or other authorised officer of the Ministry of Agriculture and Fisheries.

(a) 40 & 41 Vict. c. 68, 7 Edw. 7. c. 4 and 17 & 18 Geo. 5. c. 32.

'Minister' means the Minister of Agriculture and Fisheries.

'Plant' includes tree and shrub, and the fruit, seeds, tubers, bulbs, corms, rhizomes, roots, layers, cuttings and other parts of a plant.

'Raw vegetables' includes raw tomatoes, raw aubergines, and raw salads, but does not include mushrooms or cucumbers.

'Unhealthy' means affected, with any insect, fungus, bacteria, or other vegetable or animal organisms or any agent causative of a transmissible crop disease.

Application of Order

2. Nothing in this Order shall be deemed to prohibit or restrict the landing or transhipment in England or Wales of any plant grown in Scotland, Northern Ireland, Eire, the Isle of Man or the Channel Islands.

Prohibition against landing and restriction on transhipment in England or Wales of certain potatoes

3. (1) The landing in England or Wales of any potatoes grown in the undermentioned countries is hereby prohibited :—

the United States of America, the Dominion of Canada and European France.

(2) The transhipment in England or Wales of any of the potatoes specified in paragraph (1) of this Article is prohibited except under and in accordance with the conditions of a licence issued by the Minister or by an Inspector.

(3) In this Article 'potatoes' includes potato haulms, leaves and stalks.

Prohibition of importation of plants of sugar beet and mangold and of chrysanthemum plants

4. (1) For the prevention of the introduction of virus diseases of sugar beet and mangold the landing in England or Wales of any plant of sugar beet or mangold of the species *Beta vulgaris* Linn. (except the seeds thereof) is hereby prohibited except under and in accordance with the conditions of a licence issued by the Minister or by an Inspector.

(2) For the prevention of the introduction of the Chrysanthemum Midge (*Diarthronomyia hypogaea* F. Löw.) the landing in England or Wales of any chrysanthemum plant (except the seeds thereof) is hereby prohibited except under and in accordance with the conditions of a licence issued by the Minister or by an Inspector.

Restrictions on landing in England or Wales of certain plants and potatoes

5. (1) The landing in England or Wales of any of the plants mentioned in the First Schedule to this Order and of potatoes is hereby prohibited, unless each consignment is accompanied by two copies of a certificate of a duly authorised Official of the Phytopathological Service of the country in which the plants or potatoes were grown in the form prescribed in the Second Schedule to this Order. The inspection referred to in the certificate shall be carried out not more than fourteen days prior to the date of shipment. The original of the certificate shall be forwarded by post by the exporter to the Horticulture Branch of the Ministry of Agriculture and Fisheries, 10, Whitehall Place, London, S. W. 1, before the plants or potatoes are dispatched. Except in the case of consignments imported through the post, a copy of the prescribed certificate shall be delivered to the proper Officer of Customs and Excise at the same time as and together with the entry relating to the consignment. In the case of consignments imported through the post, a copy of the prescribed certificate shall be affixed to each package.

(2) In the case of any of the plants mentioned in the First Schedule to this Order and grown in the United States of America the certificate required by paragraph (1) of this Article shall be the certificate of an Official duly authorised by the United States Department of Agriculture.

(3) The certificate prescribed in this Article shall, except in the case of a consignment consisting wholly of potatoes, include a statement to the effect that the consignment does not contain any plant of sugar beet or mangold or any chrysanthemum plant, or any plant of the genus Ulmus or of the undermentioned genera of the order Pinaceae, *viz.*, Abies, Larix, Picea, Pinus, Pseudotsuga, Sequoia, Thuja, and Tsuga, except the seeds thereof.

(4) In the case of any of the plants mentioned in the First Schedule to this Order and grown in European France the certificate required by paragraph (1) of this Article shall include a certificate of an Officer of the French Services d'Inspection Phytopathologique which shall be in the Form A or the Form B set out in the Third Schedule to this Order.

(5) In the case of any of the plants mentioned in the First Schedule to this Order and of potatoes grown in Belgium, Germany, Luxemburg, the Netherlands, or Switzerland the certificate required by paragraph (1) of this Article shall include a certificate of a duly authorised Official of the Belgian, German, Luxemburg, Dutch or Swiss Phytopathological Service, as the case may be, which shall be, where the certificate relates to a plant, in the Form A or the Form B set out in the Third Schedule to this Order (as the circumstances may require), and where the certificate relates to potatoes, in the said Form A.

(6) The reference in Article 3 of the importation of elm trees and conifers (Prohibition) Order of 1933 [a] to the certificates prescribed in Article 4 of the Importation of Plants Order of 1933, shall be read and have

[a] S. R. & O. 1933 (No. 1011), p. 748

effect as a reference to the certificate prescribed in paragraph (1) of this Article.

(7) Paragraphs (4) and (5) of this Article shall not apply to flower bulbs, corms, tubers or rhizomes.

(8) Nothing contained in this Article shall be deemed to permit—

(*i*) the landing or transhipment in England or Wales of any potatoes of which the landing or transhipment is prohibited or restricted under Article 3 hereof;

(*ii*) the landing in England or Wales of any plant of sugar beet or mangold of the species *Beta vulgaris* Linn. or of any chrysanthemum plant of which the landing is prohibited or restricted under Article 4 hereof.

Restrictions on the landing in England or Wales of raw vegetables

6. (1) The landing in England or Wales between the twenty-first day of April and the thirtieth day of September in any year of any raw vegetables grown in European France or Switzerland is hereby prohibited unless each consignment is accompanied by a certificate of an Officer of the French Services d'Inspection Phytopathologique or of as duly authorised official of the Swiss Phytopathological Service (as the case may be) in the Form A or the Form B set out in the Third Schedule to this Order. Provided that this restriction shall not apply before the twenty-second day of May in any year to any consignment accompanied by a certificate of a duly authorised Official of the French Services d'Inspection Phytopathologique in the form set out in the Fourth Schedule to this Order.

(2) The landing in England or Wales between the first day of June and the thirtieth day of September in any year of any raw vegetables grown in Belgium, Germany, Luxemburg, or the Netherlands is hereby prohibited unless each consignment is accompanied by a certificate of a duly authorised Official of the Belgian, German, Luxemburg or Dutch Phytopathological Service (as the case may be) in the Form A or the Form B set out in the Third Schedule to this Order.

(3) The landing in England or Wales between the twenty-first day of April and the thirtieth day of September in any year of any raw vegetables grown in any country other than Belgium, European France, Germany, Luxemburg, the Netherlands or Switzerland is hereby prohibited unless each consignment is accompanied by a certificate of origin visé by a competent authority in the country of origin stating the country and place where the raw vegetables were grown.

(4) The certificates prescribed in this Article shall be delivered to the proper Officer of Customs and Excise at the same time as and together with the entry relating to the consignment.

(5) Nothing contained in this Article shall be deemed to permit the landing or transhipment in England or Wales of any potatoes of which the landing or transhipment is prohibited or restricted under Article 3 hereof.

Restrictions on the landing in England or Wales of certain raw apples

7. (1) The landing in England or Wales between the twenty-first day of April and the thirtieth day of September in any year of any cider apples grown in European France or Switzerland is hereby prohibited unless each consignment is accompanied by a certificate of an Officer of the French Services d'Inspection Phytopathologique or of a duly authorised Official of the Swiss Phytopathological Service in the Form A or the Form B set out in the Third Schedule to this order.

(2) The landing in England or Wales between the twenty-first day of April and the thirtieth day of September in any year of any cider apples grown in Belgium, Germany, Luxemburg or the Netherlands is hereby prohibited unless in the case of a consignment landed between the twenty-first day of April and the thirty-first day of May in any year each consignment is accompanied by a certificate of origin visé by a competent authority in the country of origin stating the country and place where the cider apples were grown; and in the case of a consignment landed between the first day of June and the thirtieth day of September in any year each consignment is accompanied by a certificate of a duly authorised Official of the Belgian, German, Luxemburg or Dutch Phytopathological Service (as the case may be) in the Form A or the Form B set out in the Third Schedule to this Order.

(3) The landing in England or Wales between the twenty-first day of April and the thirtieth day of September in any year of any cider apples grown in any European country other than Belgium, France, Germany, Luxemburg, the Netherlands or Switzerland, is hereby prohibited unless each consignment is accompanied by a certificate of origin visé by a competent authority in the country of origin stating the country and place where the cider apples were grown.

(4) The landing in England or Wales between the seventh day of July and the fifteenth day of November in any year of any raw apples grown in the United States of America is hereby prohibited unless each consignment is accompanied by a certificate of a duly authorised Inspector of the United States Department of Agriculture in the form set out in the Fifth Schedule to this Order.

(5) The certificates prescribed in this Article shall be delivered to the proper Officer of Customs and Excise at the same time as and together with the entry relating to the consignment.

Procedure where plants, potatoes, raw apples or raw vegetables are landed in contravention of this Order

8. (1) If plants, potatoes, raw apples or raw vegetables are landed in England or Wales in contravention of this Order, they shall forthwith be

destroyed or re-exported at the expense of the importer, unless they are otherwise disposed of in accordance with the terms of a licence issued by the Minister or by an Inspector.

(2) Such licence may provide for the examination of any such plants, potatoes, raw apples or raw vegetables, and after examination thereof, the Inspector shall give a certificate stating whether the plants, potatoes, raw apples or raw vegetables or any of them are unhealthy or not. Such plants, potatoes, raw apples or raw vegetables as are so certified to be unhealthy shall be dealt with in accordance with Article 10 hereof as the Minister or the Inspector may direct.

(3) The importer shall pay for the said certificate such fee as the Minister may determine not exceeding five pounds.

Powers of Inspector in respect of consignments of plants, potatoes, raw apples or raw vegetables

9. An Inspector, upon production if so required of his appointment or authority, may enter any premises and examine and take samples of any consignment or part of a consignment of plants, potatoes, raw apples or raw vegetables landed or suspected of having been landed in England or Wales, whether such consignment was accompanied by such certificates as are required by this Order or not. For the purpose of any examination under this Article, an Inspector may open, or require the importer to open, any package in the consignment.

Procedure in the case of unhealthy consignments of plants, potatoes, raw apples or raw vegetables

10. (1) If it appears to an Inspector at any time that any plants, potatoes, raw apples, or raw vegetables in any consignment or part of a consignment landed in England or Wales, are unhealthy, he may serve upon the importer a notice stating that such plants, potatoes, raw apples or raw vegetables have been found by him to be unhealthy, and requiring the importer, within such time as may be specified in the notice, to carry out such measures of disinfection or treatment as may be specified in the notice, or to destroy or re-export the whole or any part of the consingment.

(2) Any measure of disinfection or treatment required by a notice under this Article, shall be carried out by the importer upon whom the notice is served, under the supervision of an Inspector at a suitable place designated by the Inspector, and no part of the consignment shall be moved from the place designated without the written authority of the Minister or the Inspector.

(3) Charges for or in connection with the removal, storage, disinfection, treatment, destruction or re-exportation, required by any such notice, shall be paid by the importer on whom the notice is served.

(4) In this Article ' consignment ' includes packing materials, cases and containers,

Licences

11. Notwithstanding any provisions of this Order, any plants, potatoes, raw apples, or raw vegetables may be landed in England or Wales under and in accordance with the conditions of a licence issued by the Minister or by an Inspector.

Service of notices, etc.

12. For the purpose of this Order, a notice shall be deemed to be served on any person if it is delivered to him personally or left for him at his last known place of abode or business or sent through the post in a letter addressed to him there ; and a notice purporting to be signed by an Inspector shall be *prima facie* evidence that it was signed by him.

Information to be given as to plants, potatoes, raw apples or raw vegetables landed in England or Wales

13. Every person who has or has had in his possession or under his charge any plants, potatoes, raw apples or raw vegetables landed in England or Wales, and every person who as auctioneer, salesman or otherwise, has sold or offered for sale any such plants, potatoes, raw apples or raw vegetables shall, if so required in writing by the Minister or an Inspector, give to the Minister or Inspector all such information as he possesses as to the persons who have or have had in their possession or under their charge such plants, potatoes, raw apples. or raw vegetables provided that any information given under this Article shall not be available as evidence against the person giving same in any prosecution under this Order, except in respect of an alleged failure to comply with this Article.

Offences

14. Every person shall be liable on conviction to a penalty not exceeding ten pounds, or in respect of a second or subsequent offence to a penalty not exceeding fifty pounds who wilfully obstructs or impedes an Inspector in the exercise of his powers under this Order or who does any act in contravention of this Order or of the terms or conditions of any notice or licence served or issued thereunder, or fails to do any act which he is required to do by this Order or any such notice or licence.

This Article does not apply to the landing or attempted landing of anything in contravention of this Order, the penalty for which is provided by Section 1 of the Destructive Insects Act, 1877, as amended by the Destructive Insects and Pests Acts, 1907 and 1927.

Revocation of Orders

15. The Orders specified in the Sixth Schedule to this Order are hereby revoked.

Provided that the revocation shall not :—

(*i*) affect the previous operation of such Orders or anything duly done or suffered under such Orders ; or

(*ii*) affect any right, privilege, obligation or liability acquired, accrued or incurred under such Orders ; or

(*iii*) affect any penalty incurred in respect of any offence committed against such Orders ; or

(*iv*) affect any investigation, legal proceeding or remedy in respect of any such right, privilege, obligation, liability, or penalty as aforesaid ; and any such investigation, legal proceeding or remedy may be instituted, continued or enforced, and any such penalty may be imposed as if this Order had not been made.

Commencement

16. This Order shall come into operation on the first day of June nineteen hundred and thirty-nine.

Short title

17. This Order may be cited as the Importation of Plants Order of 1939.

In witness whereof the Official Seal of the Minister of Agriculture and Fisheries is hereunto affixed this sixteenth day of May nineteen hundred and thirty-nine.

(L. S.)

DONALD FERGUSSON,
Secretary.

FIRST SCHEDULE

All living plants and parts thereof (except seeds) for planting.

SECOND SCHEDULE

This is to certify that (Strike out words not applicable). { the living plants / a representative sample of the living plants }

included in the consignment of which particulars are given below, were/was thoroughly examined on the ..... (date) by..... .... (name) ..... a duly authorised Official of the..... ..... and found to be healthy, no evidence of the presence of any insect, fungus or pest destructive to agricultural or horticultural crops having been found in them.

This additional statement must be given for all consignments except those consisting wholly of potatoes

It is further certified that the consignment does not contain any plant of the genus Ulmus, or of the genera of the Order Pinaceae, *viz.*, Abies, Larix, Picea, Pinus, Pseudotsuga, Sequoia, Thuja, and Tsuga, or any plant of sugar beet or mangold (*Beta vulgaris* Linn.) or any chrysanthemum plant.

This additional certificate must be given for all potatoes :—

It is further certified that no case of the disease known as 'wart disease' or 'black scab' of potatoes (*Synchytrium endobioticum*) has occurred at any time on the farm or holding where the potatoes included in the consignment were grown, nor within two kilometers thereof.

(Signed).....

(Official Status).....

(Date).....

No. and description of packages.....
Distinguishing marks.....
Description of living plants or parts thereof.....
.....
Stated to be grown at.....
Exported by .....
Name and address of consignee.....
.....
Name of vessel.....
Date of shipment.....
Port of landing in England or Wales.....

THIRD SCHEDULE

Form A

This is to certify that during the 12 months preceding the date of this certificate there has been no outbreak of the Colorado Beetle in.....
..... (here insert name of country—either, Belgium, France, Germany, Luxemburg, the Netherlands or Switzerland) within a distance of 50 kilometres from the place where the living plants, potatoes, raw vegetables or cider apples (strike out words not applicable) included in the package or consignment described below were grown.

(Signed).....

(Official Status).....

(Date of issue of certificate).....

No. and description of packages.....
Distinguishing marks.....
Description of produce.....
.....

Stated to be grown at.....
Exported by.....
Name and address of consignee.....
.....
Name of vessel.....
Date of shipment.....
Port of landing in England or Wales.....

FORM B

In accordance with the authorisation contained in the General Licence No.....of the Ministry of Agriculture and Fisheries of England and Wales, this is to certify that the plants included in the package or consignment described below have been examined and have been found to be of the following species or kind.....
.....
and that the conditions prescribed in the above General Licence have been complied with.

(Signed).....
(Official Status).....
(Date).....

No. and description of packages.....
Distinguishing marks.....
Description of produce.....
.....
.....
.....
Stated to be grown at.....
Exported by.....
Name and address of consignee.....
.....
Name of vessel.....
Date of shipment.....
Port of landing in England or Wales.....

FOURTH SCHEDULE

This is to certify that the raw vegetables included in the package or consignment described below were grown in the Department of Nord or in the Department of Pas-de-Calais.

(Signed).....
(Official Status).....
(Date of issue of certificate).....

No. and description of packages.....
Distinguishing marks.....

Description of produce.....
.....
.....
.....
Stated to be grown at.....
Exported by .....
Name and address of consignee.....
.....
Name of vessel.....
Date of shipment.....
Port of landing in England or Wales.....

FIFTH SCHEDULE

This is to certify that the raw apples included in the package or consignment described below are of one of the following grades as recognised by the Department of Agriculture of the United States of America—

'U. S. Fancy'

'U. S. (No. 1)'

'Extra Fancy'

'Fancy'

(Signature).....

(Official Status).....

(Date).....

Description of consignment

Number and nature of packages.....
Distinguishing marks.....
Variety of apples.....
Name and address of consignee.....
.....
Name of vessel.....
Date of shipment.....
Port of shipment.....

SIXTH SCHEDULE

The Importation of Plants Order of 1933.[a]
The Importation of Plants (Amendment) Order of 1934. [b]
The Importation of Plants (Amendment) Order of 1935. [c]
The Importation of Plants (Amendment) Order of 1936. [d]
The Importation of Plants (Amendment No. 2) Order of 1936. [e]
The Importation of Plants (Amendment) Order of 1937. [f]
The Importation of Plants (Amendment) Order of 1938. [g]

C. D. DESHMUKH,
Joint Secretary to the Government of India.

NOTIFICATION No. F. 7-6/39-A, DATED THE 29TH JUNE 1939 ISSUED BY THE GOVERNMENT OF INDIA IN THE DEPARTMENT OF EDUCATION, HEALTH AND LANDS

An Order similar to that published with this Department notification No. F. 7-6/39-A, dated the 15th June 1939, has been issued by the Department of Agriculture for Scotland.

C. D. DESHMUKH,
Joint Secretary.

THE THIRD SESSION OF THE INDIAN STATISTICAL CONFERENCE, MADRAS, 1940

The third session of the Indian Statistical Conference will be held in Madras in the first week of January 1940. It has been provisionally arranged that the Conference will be opened by His Excellency the Governor of Madras on the 3rd January 1940. On the invitation of the Mysore University arrangements have also been made for holding a special session in Mysore possibly on the 6th and 7th January.

In Madras the work of the Conference will be carried on in active co-operation with the Indian Science Congress as in previous years. Arrangements have been made for joint meetings with the Sections of Mathematics, Physics, Agriculture, and possibly the Section of Medicine and Public Health. There will also be a special session on Economic Statistics.

(a) S. R. & O. 1933 (No. 558), p. 735.
(b) S. R. & O. 1934 (No. 684) I, p. 458.
(c) S. R. & O. 1935 (No. 1225), p. 399.
(d) S. R. & O. 1936 (No. 313) I, p. 871.
(e) S. R. & O. 1936 (No. 1288) I, p. 874.
(f) S. R. & O. 1937 (No. 197), p. 659.
(g) S. R. & O. 1938 (No. 517) I, p. 1143.

The Hon'ble Mr. V. V. Giri, Minister for Labour and Industries, Government of Madras, has kindly agreed to preside over a special discussion on 'Labour Statistics'.

The Government of India, different Provincial Governments, many of the important Indian States and Universities in India have given their official recognition to the Conference and are expected in many cases to send official delegates.

The subscription for ordinary members of the Conference is Rs. 5 and for Associate and Student members Rs. 3 and Re. 1 respectively. Members of the Indian Statistical Institute will be entitled to become Ordinary members of the Conference without payment of any additional subscription. Ordinary members of the Conference will have the right to contribute papers, to attend all scientific and other meetings of the Conference and to receive, free of charge, all publications relating to the conference.

Papers and scientific contributions should be sent so as to reach the Honorary Secretary, Indian Statistical Institute, Statistical Laboratory, Calcutta, before the 15th December 1939.

Enquiries in regard to local arrangements and accommodation should be addressed to the Local Secretaries, Prof. P. J. Thomas, University of Madras, or Prof. K. B. Madhava, University of Mysore.

ALGAL-FLORA OF PADDY FIELD SOILS OF THE UNITED PROVINCES, I

WHILE commenting on my note 'On Algae Found in Soil Samples from an Alluvial Paddy Field of Faridpur, Bengal' published in *Science and Culture*, Vol. I, No. 5, p. 298, Mr. R. N. Singh has made some observations in his article 'An Investigation into the Algal-flora of Paddy Field Soils of the United Provinces, I' published in this Journal, Vol. IX, Part I, February 1939, pp. 55-77. As a reply to these criticisms, I might state that the object of the investigation was not to bring out those algae alone which can resist prolonged desiccation, but to identify all the algal types actually contained in the two samples, collected from the surface of a paddy field of Faridpur, as desired by the Imperial Council of Agricultural Research. Moreover, the material available did not offer the opportunities for studying the soil algae from various depths with regard to their economy in soil, etc.

The role of algae in fixing nitrogen in soil was not also the subject matter of my investigations. No special effort was, therefore, made to eliminate the bacteria, although as pure cultures as possible were made.

Although diatoms occur universally, none of them was found in any of my cultures. No other member of the green algae, except *Pleurococcus Naegelii Chodat*, made its appearance in the culture media used. The name for this species was given as it could be ascertained from available references at the time of sending out the note for publication. (J. C. Banerji).

REVIEW

The Soils of Central Africa, in particular of the Belgian Congo, Vol. I. The Lower Congo. By Professor J. BAEYENS. Published by The National Institute for Agronomical Studies of Belgian Congo, Brussels. Price Fr. 150. 1938

IN his preface to the book Sir John Russell refers to the rapid progress that has been made in agronomy as a result of the recognition of pedology as an independent branch of science and also congratulates Professor Baeyens on his successful researches. The book is comprehensive and the reviewer has no hesitation in stating that it is a valuable contribution to pedological studies. The object and scope of the investigations undertaken by the author will be evident from the following quotation :—

'The pedological study of the soils in the Belgian Congo may be considered either from a scientifical or a practical point of view. The first must logically take precedence over the second. In order to obtain in a short time the practical results requested by State or private undertakings both studies were performed simultaneously. The first part of the book gives a general outlook on the pedological properties of the soils in the Lower Congo. The second part deals with their agricultural properties not directly on the matter of manure requirements but of their original value with their subsequent classification based on a general scale, including: very fertile, fertile, wanting, or bad soils. This is regarding the most important crop plants actually grown in the Lower Congo'.

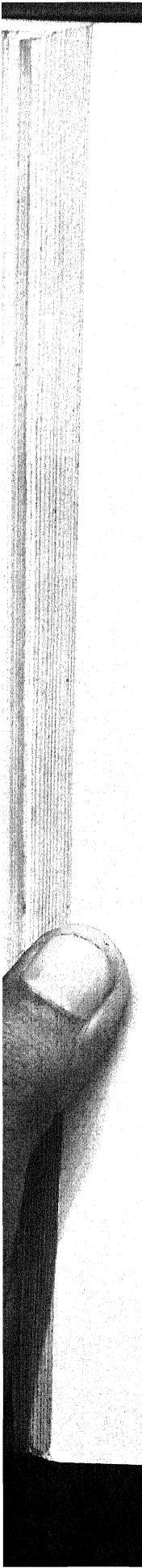
In order to organize a soil survey service according to modern pedological methods, research work was started in Central Africa in 1934-35. The results are discussed in the book under the following sections :—

The factors dealing with the fertility of the soil in the Lower Congo; the importance of a study of profiles—the technique used in their study and description of some typical profiles; the mechanical properties and the structure of the soils; the water relationship—static and dynamic; chemical and colloidal properties; the organic matter and biological activity; the climate and the pedological climate. These are followed by a section dealing with the classification and cartography of the soils. The next chapter deals with the genesis of the soils.

The above chapters form Part I of the book.

In the second part the principles on which a scale of pedological fertility can be based are dealt with. This is followed by discussion on the suitability of soils for the principal crops of the country, viz. cocoa, coffee, etc. These are followed by other sections dealing with the relative agricultural values based on pedological characteristics of the soils of the country.

The book contains 11 maps, 47 tables and a number of very interesting figures showing the topography, vegetation, typical profiles, etc. [J. N. M.].



RECENT PUBLICATIONS OF THE IMPERIAL AGRICULTURAL BUREAUX

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IV. Obtainable from the Imperial Bureau of Plant Breeding and Genetics (for Crops other than Herbage), Plant Breeding Institute, School of Agriculture, Cambridge

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V. Obtainable from the Imperial Bureau of Pastures and Forage Crops, Welsh Plant Breeding Station, Agricultural Buildings, Alexandra Road, Aberystwyth, Wales

Journal

	s.	d.
Herbage Abstracts—		
Vol. 8, 1938; Vol. 9, 1939, each	*25	0
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* 20*s.* to subscribers in the British Commonwealth.
† A reduction of 5*s.* will be given when Herbage Abstracts is also ordered.
‡ Joint publication of Bureaux of Soil Science and Pastures and Forage Crops.

VI. Obtainable from the Imperial Bureau of Horticulture and Plantation Crops, East Malling Research Station, East Malling, Kent

Journal

	s.	d.
Horticultural Abstracts. A quarterly abstract publication of current horticultural literature.		
Vol. I. First issued, March, 1931. No. 2, single copy . . .	1	6
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Proceedings of First Imperial Horticultural Conference, 1930—

Part II. Papers on the application of Science to Horticulture. 1931. 2s. Out of print, but the following separates are available, each . 0 3

Field Experiments in Certain Tropical and Sub-Tropical Crops in West Africa (Dr. E. J. Martin and W. H. Beckett).

Fruit Products and Associated Problems (Professor B. T. P. Barker).

Some Physiological Considerations in Horticulture (Professor V. H. Blackman).

Plant Physiological Work in the Tropics: Some of the Problems, with Special Reference to Cocoa and some Possible Lines of Attack (Dr. T. G. Mason and Dr. E. J. Maskell).

Soil and Survey Work as a Basis for Fruit Production in Irrigated Areas (Professor A. J. Prescott).

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The Behaviour and Diseases of the Banana in Storage and Transport (Dr. C. W. Wardlaw and Dr. L. P. McGuire).

Problems of Storage and Transport (E. A. Griffiths).

Fruit Transport Problems in Canada (R. L. Wheeler).

The Infection and Invasion of the Apple Fruit by Fungi in Relation to the Problem of Storage (Dr. A. S. Horne).

Experiments on the Preservation of Citrus Fruits (Dr. W. J. Young and F. M. Read).

* 20s. a volume or 5s. a number to subscribers in the British Commonwealth.

Other Publications—

Index to Volumes I—X of the *Journal of Pomology and Horticultural Science*, 1933. Compiled by Bureau, published by the Editors of the *Journal of Pomology and Horticultural Science.*

	s.	d.
Available from the Bureau	5	0

Fruit growing in the Empire, standardisation of horticultural material with special reference to root stock, 1927. R. G. Hatton, being un-numbered Empire Marketing Board Publication (Free).

VII. Obtainable from the Imperial Bureau of Animal Breeding and Genetics, Institute of Animal Genetics, University of Edinburgh, Kings' Buildings, West Mains Road, Edinburgh

Journal	s.	d.
Animal Breeding Abstract (quarterly) from Vol. 6 (1938)—		
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Also obtainable from the Bureau:		
The Genetics of the Horse, 1930. Reprinted from 'Bibliographia Genetica'	0	6
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VIII. Obtainable from the Imperial Bureau of Agricultural Parasitology (Helminthology), Institute of Agricultural Parasitologoy, Winches Farm Drive, Hafield Road, St. Albans, Herts

Journal	s.	d.
Bibliography of Helminthology. For the years 1930, 1931, 1932, 1933— Library Edition, bound in cloth per volume	10	6
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Helminthological Abstracts (1932 onwards). Issued in five or six parts per volume, each volume covering the literature published in a single year. Subscription per volume (payable in advance) . . .	30	0
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[NOTE.—From Vol. III (1934) this journal incorporates the *Bibliography of Helminthology* and is published by the Bureau instead of by the *Journal of Helminthology*.]

Other Publications		
(*j*) Helminth parasites of New Zealand. By May R. Young, 19 pp.	4	0
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Publications issued by the Imperial Institute of Entomology, 41, Queen's Gate, London, S. W. 7

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